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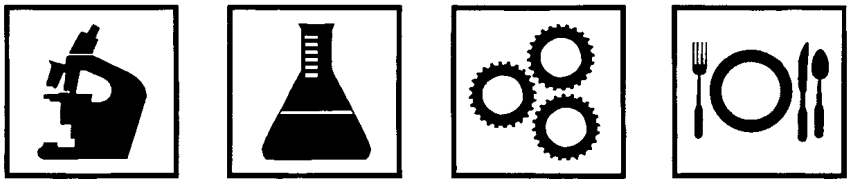

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ABSTRACTS

This is a collection of the abstracts from IAFP 2017, held in Tampa, Florida.

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6200 Aurora Avenue, Suite 200W Des Moines, Iowa 50322-2864, USA
+1 800.369.6337 +1 515.276.3344 Fax +1 515.276.8655
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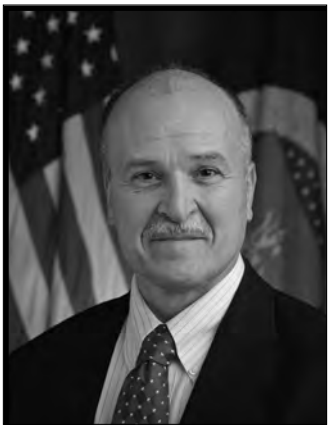
IVAN PARKIN LECTURE ABSTRACT

The Anthropologist, the Chef, and the Kitchen Sink



Jose Emilio Esteban
Science Advisor
United States Department of Agriculture
FSIS-OPHS-EALS
Athens, Georgia

Food safety today is not the same as it was yesterday or a year ago or even a decade ago. How we interact within and between academia, industry, and government has to change and adapt. Pathogens change; we adjust by creating new interventions. Biocides are developed and drug residues are introduced into our food supply; we find better ways to decontaminate. Constant changes in hazards require us to generate new detection and characterization technologies in an endless attempt to detect at lower levels with faster speed and with more accuracy. Where does this cycle end? In this lecture, I will share two perspectives – that of an anthropologist and that of a chef, both addressing the same goal: to have enough food, feed, and fuel, to sustain an ever-growing (and aging) population.

When was the last time you had time to think about how we got to here? What is considered food today may not have been "food" a few years ago. What is normal for one consumer group may be considered strange for another. Today's level of detection for an analytical method was only considered theoretical a few years ago. Remember life without a cell phone? Remember life without the internet? Pathogens that could be easily neutralized are now resistant and that resistance is now a permanent part of the genetic possibilities for the foreseeable future.

We may all walk different paths and we will all have intermediate stops; however, we are all headed in the same general direction. The IAFP Annual Meeting is the one occasion where industry, academia and government representatives from around the world assemble to exchange information. Relationships are forged, lifelong partnerships are made, and the seeds of change are planted. We all have one goal in mind — food safety. Unless we try to understand where we came from and where we are, it's impossible to know where we want to be.

The anthropologist view will help us understand characteristics of consumers, behaviors, and preferences. Only by understanding this can we move forward to where we want to be. The chef perspective will then give us a sense of reality for today and instill creativity for where we can go. Hope you enjoy a personal perspective of the world through metaphors.

JOHN H. SILLIKER LECTURE ABSTRACT

Food Allergies: A Public Health Dilemma – How Did We Get Here? Where are We Going?

Steve L. Taylor
Professor & Co-Director
Food Allergy Research & Resource Program
Department of Food Science & Technology
University of Nebraska
Lincoln, Nebraska

Food allergies have been described in medical literature for over 100 years. But the first 75 years of that history were fairly quiescent. Beginning in about 1990, food allergies began to emerge as an important public health issue. The prevalence of food allergies began to rise and rise dramatically, especially among infants and young children. Food allergies began to be recognized as a potentially severe, life-threatening condition. And, the potency of certain foods as allergens – "it only takes one bite" – became known. As the awareness and seriousness of food allergies emerged, the food industry struggled because the most commonly allergenic foods and especially milk, egg, soy and wheat were almost ubiquitous in food processing facilities. The industry had no tools or ability to assess the risk. The public health authorities similarly lacked tools and knowledge but were obliged to take a conservative approach to protect food-allergic consumers.

In the intervening 25 years, enormous progress in our understanding of food allergies has been made. We are beginning to understand the reasons for the increasing prevalence of food allergies. The path toward prevention of the development of food allergies among infants and young children seems clear. While a cure for food allergies still seems elusive, clinicians are investigating immunotherapy strategies that promise to curtail the potency and severity of food allergies. On the public health side, improved labeling regulations have been implemented in the U.S. and several other countries; packaged foods are safer for those with food allergies than they have ever been. The Food Safety Modernization Act identifies food allergens as a recognized public health hazard and mandates the development of preventive allergen controls. The industry now has the analytical tools needed to identify allergen hazards and assess the effectiveness of allergen control approaches. Quantitative risk assessment is emerging as a decision-making approach to guide labeling and industrial allergen management.

We may not put this public health issue completely behind us over the next 25 years, but I do think that we will lessen the public health impact of food allergies considerably.

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Symposium Abstracts

S01 STEC Regulation: What is Needed in Global Food Trade?

PETER GERNER-SMIDT: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
PETER FENG: *U.S. Food and Drug Administration, College Park, MD, USA*
IAN JENSON: *Meat & Livestock Australia, North Sydney, Australia, Australia*

In 1998 USDA-FSIS declared *Escherichia coli* O157:H7 and, then in 2012, six non-O157 shiga toxin-producing *E. coli* serogroups (O26, O45, O103, O111, O121 and O145), known as non-O157 STECs, to be adulterants in non-intact raw beef products (zero tolerance policy). The surveillance program, put in place by FSIS, has shown the prevalence of these *E. coli* O157:H7 and the non-O157 STECs; and the risk mitigation strategies employed by U.S. processors have been effective. Beside US beef processors, all beef exporting countries to the U.S. (Australia, New Zealand, and, more recently, Brazil) have also been impacted by this regulation and have included these food hazards in their food safety management plans and in their food testing programs.

In the meantime, other countries have become concerned by food poisoning due to STECs in produce and dairy matrices. In 2011, Germany incurred the largest ever reported outbreak of STEC O104:H4 illness, linked to the consumption of fenugreek sprouted seeds. As a consequence, the EU, in 2013, issued microbiological criteria for sprouts, including recommendations for STEC testing in sprouts (O157, O26, O103, O111, O145 & O104:H4) and the use of the ISO TS 13136 STEC testing standard. Following on from such, countries have, also, increased their testing for STECs in beef and dairy products. Numerous products have been incriminated in product recalls or border rejections.

However, official microbiological criteria for STECs have yet to be defined by the EU for these food products and the basis for past actions appears to have been hazard-based rather than risk-based. Such actions and the rapid move towards molecular gene sequence-based detection methods with an as yet incomplete understanding of the relationship between the presence of genes and risk threaten international trade. This symposium will illustrate the current understanding around STECs in foodborne outbreaks, including data on surveillance programs among different food types; and will highlight how all of that translates to international food trade. Speakers from U.S. and Australia/NZ will present some of their key data linked to STEC and show the understanding and expectations of their respective countries.

S02 Antibiotics in Pre-harvest Production and Associated Risks to Food

ASHLEY PETERSON: *National Chicken Council, Washington, DC, USA*
LIZ WAGSTROM: *National Pork Producers Council, Urbandale, IA, USA*
PAUL MORLEY: *Colorado State University, Fort Collins, CO, USA*

Antibiotics in food production have come under great scrutiny. This symposium will look at different elements impacting food safety risks in the production chain and the role antibiotics play. Presenting different topics from multiple markets provides an overall understanding of the various elements involved in pre-harvest that impact food safety. Each topic will be presented by a different commodity group allowing visibility of problems and solutions across animal protein production. Topics will help demonstrate the impact of antibiotic removal, layered programs to reduce risks, and the necessity of research as a part of a complete pre-harvest food production system. The discussion will demonstrate the need to balance risks in production systems, some examples being: output, zoonotic disease, antimicrobial resistance, disease detection, microbial loads, and residues. Topics are:

1. What changes, if any, are seen in food products when antibiotics are removed from production in the poultry industry;
2. How antibiotic programs in production are reducing risk in the swine industry; and)
3. The importance of data based decisions to make changes in production systems, like the beef industry, with longer life cycles.

S03 Virulence Factors and Host Susceptibility of Foodborne Pathogens

DAVID BEAN: *Federation University Australia, Ballarat, Australia, Australia*
ROY BETTS: *Campden BRI, Gloucestershire, United Kingdom, United Kingdom*
JANELL KAUSE: *U.S. Department of Agriculture–FSIS, Washington DC, DC, USA*
TRUDY WASSENAAR: *Molecular Microbiology and Genomics Consultants, Zotzenheim, Germany, Germany*
KUMAR VENKITANARAYANAN: *University of Connecticut, Storrs, CT, USA*
MONICA PONDER: *Virginia Tech, Blacksburg, VA, USA*

Foodborne illnesses continue to be a major public health concern. All members of a particular bacterial genera are often treated by public health and regulatory agencies as being equally pathogenic. However, even within species, virulence factors vary to the point that some isolates may be highly pathogenic, whereas others may rarely, if ever, cause disease in humans. Hence, many food safety scientists have concluded that a more appropriate characterization of bacterial isolates for public health purposes could be assessing virulence factors. While individual genetic characteristics of the foodborne pathogen are influential in determining virulence, the ability of these bacteria to induce illness in a human may, also, be greatly affected by numerous host susceptibility factors. Some of these factors include age of the host, current immune status, type and amount of food consumed, and host cell genetic makeup. The virulence of particular pathogens may also increase in the presence of certain antibiotics and dietary supplements (e.g., acid neutralizers and high fiber diets). Attempting to establish regulatory guidelines, based on all the above-mentioned factors, is extremely challenging and has led to differences among national regulatory agencies in their standards, such as the U.S. limit for *Listeria monocytogenes* of 0.04 CFU/g in food versus the European limit of 100 CFU/g in selected cases. Attempts have also been made to mitigate foodborne pathogen illnesses by means of probiotics, prebiotics, and synbiotics. This symposium will address these topics to provide a comprehensive treatise of the complex factors that influence the virulence of foodborne pathogens in the human host.

S04 Developments in Mycotoxin Research: From Methodology to Prevention

ROB SAMSON: *CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands, Netherlands*
LUDWIG NIESSEN: *Lehrstuhl für Technische Mikrobiologie, Freising, Germany, Germany*
GIANCARLO PERRONE: *Institute of Sciences of Food Production National Research Council, Bari, Italy, Italy*
NARESH MAGAN: *Cranfield University, Shrivenham, United Kingdom, United Kingdom*
DOJIN RYU: *University of Idaho and Washington State University, Moscow, ID, USA*
EMILIA RICO-MUNOZ: *BCN Research Laboratories, Inc., Rockford, TN, USA*

Knowledge of the mycobiota of foods is essential to the understanding and prevention of spoilage. In addition to causing spoilage, growth of filamentous fungi in foods can result in the production of mycotoxins and other secondary metabolites, which may impact human health. Not all mycotoxigenic mold species will produce mycotoxins when growing on a particular food. The production of mycotoxins is not only substrate specific, but it also depends on other factors. Understanding the interactions of the several factors that affect the production of mycotoxins is fundamental to determine health risks associated with mold-spoiled foods and beverages.

In this symposium, different aspects of the production of mycotoxins in foods and beverages will be discussed. The symposium will start with a general overview of the different aspects of mold spoilage and mycotoxin production. The associated mycobiota of foods and the risk of mycotoxin production, as well as factors that influence mycotoxin production will be discussed. The latest methodology for the detection of mycotoxins will be presented. Mycotoxin production during mold spoilage of dairy products and fermented sausages will, also, be covered. The symposium will end with recommendations on how to prevent mold spoilage and mycotoxin production in foods and beverages.

S05 Pathogen Contamination at Retail: What are the Next Steps?

KRISTINA BARLOW: *U.S. Department of Agriculture–FSIS, Washington D.C., DC, USA*
GLENDA LEWIS: *U.S. Food and Drug Administration–CFSAN, College Park, MD, USA*
HILARY THESMAR: *Food Marketing Institute, Arlington, VA, USA*

Contamination from pathogens at retail continues to cause foodborne illness. Retail facilities are subject to pathogen contamination from the environment, incoming raw products, and employee handling. Recently, the Food Safety and Inspection Service (FSIS) has issued new requirements for retailers to keep grinding logs and has started performing surveillance in the retail deli to determine if retailers are following recommendations to control *Listeria monocytogenes*. In addition, the U.S. Food and Drug Administration (FDA) Food Code contains recommendations for controlling pathogens in food. However, questions still remain about the best way to control pathogens at retail. This symposium will characterize the current status of requirements and recommendations for pathogen control at retail and identify next steps that retailers can take moving forward.

S06 Perishable Foods Delivered to Homes via Common Carriers: Safe or Sorry?

MELANIE ABLEY: *U.S. Department of Agriculture, Washington, DC, USA*
WILLIAM HALLMAN: *Rutgers University, New Brunswick, NJ, USA*
FRANK YIANNAS: *Walmart, Bentonville, AR, USA*

The rise of e-commerce and ubiquity of online ordering has dramatically increased the prevalence of home-delivered perishable foods. These foods include groceries, fresh produce, meat, poultry, seafood, cheese, meal preparation kits and prepared meals. As demand grows, both providers and consumers can benefit from developing a better understanding of the particular food safety requirements for online ordering of food and the food safety challenges involved with shipment of perishable foods. This symposium will bring together food safety experts from various backgrounds with extensive experience in the area of home delivered food products. A speaker from the U.S. federal government sector will provide insights into the regulations applicable to home-delivered foods in the U.S. and abroad, helping to provide clarity to the regulatory framework in which providers must operate. Second, a speaker from the academic sector will present research findings and case studies on home-delivered food products, highlighting food safety risks and identifying provider and consumer knowledge gaps in this area. Finally, an industry speaker will discuss approaches to tackling the unique technical, logistical, and regulatory challenges of home-delivered foods. Attendees should gain a better understanding of leading practices for both providers and consumers of home-delivered perishable foods, including packaging and shipping guidelines, handling and storage guidelines, and means for effective communication of food safety risks.

S07 Latin America: Issues and Initiatives for Food Safety

MARISA CAIPO: *Food and Agriculture Organization of the United Nations, Santiago, Chile, Chile*
JAIRO ROMERO: *Jairo Romero y Asociados SAS, Bogota, Colombia, Colombia*
MARIA TERESA DESTRO: *bioMérieux, Inc., São Paulo, Brazil, Brazil*

From innovative Incan terraces on steep mountain slopes, where potatoes and peppers flourish in Peru's iconic Andes range, to the vast pampas of Argentina, where the legendary gauchos herd cattle on horseback, to prolific coffee plantations in Brazil's subtropical southeastern states, and to equally unique destinations in between, South America abounds with places where a tremendous variety of great food is produced for locals and the world. Moreover, food is produced in different seasons than in Northern hemisphere continents, and this contributes favorably to the consistency of foreign food supplies year-round. South American countries export a wide variety of foods to the U.S., including fresh fruits, salmon, beef, wine, and coffee. The U.S. imported a whopping $18.509 billion worth of agricultural and fish products from South America in 2014, according to U.S. Census Bureau trade data compiled by USDA. The World Trade Organization reported that South and Central America exported a combined US$ 217 billion in agricultural products in 2013.

Market access throughout the world and brand protection have been the major drivers of food safety in South America; and this has specifically led to implementation of new food legislation, as well as modernization of food safety inspection agencies to comply with international requirements and guidelines. As a result there are more food safety experts, better laboratories, more experienced food safety authorities, better rulemaking processes, and more training on food safety and quality than ever before. Yet, challenges remain. In Central America, issues include water quality, agricultural research and development limitations, hygiene challenges, lack of refrigeration, cross contamination, and improper food handling and preparation. This symposium will provide a forum to share information on food safety challenges and initiatives in Latin America.

S08 FDA Food Import Entries and Refusals: Finding Meaning within the Data

JEFFREY READ: *U.S. Food and Drug Administration, College Park, MD, USA*
JEAN BUZBY: *U.S. Department of Agriculture, Economic Research Service, Washington, DC, USA*

According to USDA trade data, the United States is importing 4–5% more food every year. Within a larger and more diverse food supply, FDA is finding new and different hazards in food; so, it is more important than ever to understand the composition of the U.S. food supply. The U.S. Food and Drug Administration (FDA), as part of its mission to protect consumers, gathers information on the origin and types of food being imported and makes entry decisions after applying a risk-based decision making process. The data FDA gathers provides a rich and unique view of what foods Americans are consuming and the regulations that food producers are struggling to meet in order to gain access to the U.S. market. While FDA gathers the data, it does not always have enough experts or processes to thoroughly analyze import data. Both industry and other government stakeholders, therefore, have attempted to mine publically available information on FDA's rejections of imported food in order to better understand patterns of regulatory non-compliance and potential risk to consumers. This session will report on recent research outcomes regarding FDA data on entries and refusals of

imported food shipments. The purpose of this session will be to highlight methods and findings of recent research in this area. Presenters will have the opportunity to suggest ways in which FDA could improve its data collection and publication to facilitate more meaningful research. Challenges include the quality and detail of publically available FDA imports data, as well as the non-random nature of FDA's import sampling programs. Finally, ways in which this data can be used by industry and regulators to improve food safety will be discussed. Audience members will leave the session with a better understanding of the challenges facing regulators, along with ideas for future collaboration among researchers.

S09 All You Wanted to Know about Antimicrobial Hand Sanitizers and Were Afraid to Ask

LEE-ANN JAYKUS: *North Carolina State University, Raleigh, NC, USA*
PRANVERA ICONOMI: *FDA-CDER, White Oak, MD, USA*
PETE CARLSON: *Ecolab Inc., St.Paul, MN, USA*

Hand sanitizers are important for interrupting the transmission of bacteria and viruses through hands. This symposium will bring together experts from academic, regulatory, and industrial arenas to provide comprehensive overviews on the current status on hand sanitizers for the public health safety. More specifically, the first presentation will focus on the most recent research findings, as far as controlling bacterial spores and the appropriateness of model viruses (human norovirus) for testing anti-viral efficacy as it relates to our food safety needs. The criteria for the properties and efficacy of hand sanitizers (consumer/healthcare) will be highlighted from an FDA-regulatory prospective. Last, but not least, the challenges in fulfilling defined efficacy data gaps will be discussed; as well as the processes for validating test methods to real-world outcomes from an industry perspective will be presented.

S10 Developing Evidence-based Recommendations to Improve Consumer Safe Food-handling: International Approach

IAN YOUNG: *Ryerson University, Toronto, ON, Canada, Canada*
SANJA ILIC: *Ohio State University, Columbus, OH, USA*
ELLEN W. EVANS: *ZERO2FIVE Food Industry Centre, Cardiff, United Kingdom, United Kingdom*

Consumers frequently underestimate risks of acquiring foodborne illness due to food consumed and prepared at home. While they may be aware of recommended food safety practices, this knowledge does not always correlate with adoption. Psychosocial factors (e.g., self-efficacy, subjective norms) may be important predictors of consumers' safe food handling. In addition, due to unique characteristics, some marginalized populations require targeted approaches to achieve the behavior change. Theories of behavior change (Theory of Planned Behavior, Health Belief Model, and Stages of Change Transtheoretical Model) provide structured and evidence-based frameworks for describing different psychosocial factors influencing behaviors.

This symposium will provide attendees with understanding of how psychosocial constructs and behavior-change theories can guide the development of effective evidence-based, targeted educational interventions to improve consumer food safety. The first presentation will describe a systematic review and meta-analysis of research studies investigating the psychosocial determinants of consumer safe food handling. The results will indicate which constructs, rooted in different behavior-change theories, are most consistently associated with behaviors, including which factors contribute to differences in findings across studies. The second presentation will provide an international perspective on the methods and measures used in consumer research. Social desirability biases and the Hawthorne effect will be explored. The relationships between consumer food safety knowledge, attitudes, and self-reported and observed behavior will be explored; and the microbial contamination outcomes of behaviors obtained using an innovative model domestic kitchen will be presented. The third presentation will discuss factors affecting behaviors in developing countries. Self-reported and observed practices among low-income mothers/caregivers of children 6–24 months receiving nutritional interventions in Tanzania and the strategies to improve food safety of homemade baby foods will be presented. Domestically, behaviors in food-insecure (low availability and accessibility of food) cancer patients and approaches to address the high food safety risk coping mechanisms will be discussed.

S11 Microbial Food Safety in Small to Medium-size Farming Systems: Risks and Mitigation Strategies

STEVE WARSHAWER: *Beneficial Farms CSA, Santa Fe, NM, USA*
CHARLES GERBA: *University of Arizona, Tucson, AZ, USA*
RICHARD BAINES: *Royal Agriculture University, Gloucestershire, United Kingdom, United Kingdom*

The purpose of this symposium is to build on the success of our roundtable session, last year, entitled "A Real-World Conversation about Food Safety and Microbial Quality of Sustainable Diversified Farming Systems". We received numerous questions and action items during and after the round table. From this information, six topics that impact farmer implementation of the produce safety rules and knowledge gaps within these farming systems impacting farmer rule compliance will be addressed. This symposium will address the following topics:

1. Risks associated with proximity of small animal herds to fruit and vegetable production systems;
2. In-field human pathogen die-off in raw manure following the NOP standard;
3. Small to medium farmers perspective on microbial risks and FSMA regulations within their farming operations;
4. Impacts of riparian areas and buffer zones on wild life corridors and fresh produce safety;
5. Antimicrobial resistance within small and medium size farming systems; and
6. Risk assessment in small to medium size farming systems.

S12 Urban Agriculture/Farming and Food Safety

PATRICIA MILLNER: *U.S. Department of Agriculture–ARS, Beltsville, MD, USA*
MICHELLE SMITH: *U.S. Food and Drug Administration, College Park, MD, USA*
JAMES RATKE: *Urban Produce Farms, West Chicago, IL, USA*

Innovative allocation and repurposing of urban land, buildings, and resources for agricultural production of fresh produce is rapidly emerging as a community-based approach to enhance food availability in urban cities. Urban agriculture usually involves high intensity production in both indoor and outdoor environments. Hydroponic systems utilizing multi-tiered plant growing systems are commonly used in urban farms. Increasingly, aquaponic systems are being employed to produce fresh fruits and vegetables using the wastewater generated from an aquaculture system. The aquaponic systems use the nutrients in the recirculating aquaculture water as the plant fertilizer for the growth of fresh produce.

Food safety risks are invariably present in a mixed-commodity food production setting; yet many of these risks remain unknown or under-researched. Information is needed regarding the risk from contaminated seeds or from irrigation water that has been recirculated and reused for extended periods of time. Little or no information is available on the efficacy of current control strategies to prevent cross-contamination or prevent the persistence and

survival of foodborne pathogens in these systems. Urban farms are usually owned by small to medium size enterprises or non-profit organizations that do not necessarily have the expertise or tools to address food safety issues and are in need of guidance with regards to hazard analysis, implementation of preventive controls, and regulatory compliance.

This symposium will provide an overview of production practices currently employed in urban agriculture/farming, summarize currently available food safety data, and discuss potential food safety risks that may be associated with these food production systems and practices. How the requirements outlined in the recently issued produce safety rule apply to urban farms will be discussed. The symposium will also feature perspectives from urban farm operators discussing the challenges in identifying potential food safety hazards and in meeting regulatory requirements.

S13 Global Dairy Indicators (Coliform vs. *Enterobacteriaceae* vs. Other Indicators): Their Value, Regulatory Impact and Effect on Global Trade

ALLEN SAYLER: *EAS Consulting Group, Alexandria, VA, USA*
MIEKE UYTTENDAELE: *Ghent University, Ghent, Belgium, Belgium*
DEON MAHONEY: *Dairy Food Safety Victoria, Melbourne, Australia, Australia*

Indicator organisms are used in the dairy industry to assess the microbial quality of raw milk, the effectiveness of pathogen control strategies, and the presence of unsanitary conditions in the processing environment. Although a range of organisms have been proposed as indices of dairy safety and quality, historically, the indicator organisms (or rather groups of organisms) of choice have been either coliforms, *Enterobacteriaceae*, or other indicators. Why would the indicator organisms of choice for dairy differ from country to country? It is not the company or industry who is choosing the group of organisms they wish to test. The dairy industry in most countries is quite heavily regulated, so the choice is, generally, based on regulation. In this session, we will first be provided with a breakdown and overview as to which areas of the globe choose coliform, *Enterobacteriaceae*, or other Indicators and why. We will, then, hear from regulators located in two regions of the globe. Each will provide an overview of how the indicator group of choice, in their region, has been chosen and shown to be effective.

S14 Strategies for Effective Hygienic Zoning

DUANE GRASSMANN: *Nestle USA, Solon, OH, USA*
POLLY COURTNEY: *General Mills, Inc, Golden Valley, MN, USA*
DOUG CRAVEN: *Hormel, Austin, MN, USA*

Hygienic zoning is used to prevent transfer of contamination from potentially contaminated areas to those where food products are exposed during manufacturing. Hygienic zoning strategies vary depending on product manufacturing steps, facility layout, personnel and equipment traffic patterns, and other factors. Although hygienic zoning can contribute greatly to ensuring safe food, many companies have not yet, fully, implemented it. Implementation of hygienic zoning may be delayed due to difficulty with physical separation of zones, establishment of practical procedures for personnel transitioning between hygienic zones, or understanding of concepts, tools, and techniques associated with hygienic zone controls. This symposium includes speakers from several companies, manufacturing different types of food products, who will explain how their hygienic zones are set up and how the barriers (physical and procedural) between those zones have been implemented to reduce likelihood of environmental contamination.

S15 The Importance of Sample Preparation for Microbiological Analysis: Anything That Begins Badly, Ends Worse

DAVID TOMAS FORNES: *Nestlé, Lausanne, Switzerland, Switzerland*
GEOFF BRIGHT: *World Bioproducts, Bothell, WA, USA*
PAUL MORIN: *U.S. Food and Drug Administration, Jamaica, NY, USA*

Although technological advances in the laboratory hold great promise for rapid diagnosis of food samples, the first step in analysis of foods aims to process the sample for downstream analysis. The challenges to sample preparation have circulated throughout the food analytical world for a long period of time. These include low numbers of targeted pathogens in foods, non-culturable microbes, such as viruses, and the decision to enrich, where possible, and to select the most appropriate media for additional growth, followed by plating on solid media that allows isolation of the pathogen.

This symposium will present current efforts to harmonize sample preparation, identify the issues that hamper successful isolation or detection of pathogens, and an insight of what occurs in the development of media, specifically for sample preparation. Bringing together like minds will, at least, keep the discussion progressing to develop efficacious, validated, and accepted sample preparation protocols that can be applied worldwide. Food safety is a global fact and as the title of this symposium implies, anything that begins badly, probably ends with bad consequences.

S16 Are Culture Methods Obsolete?

BYRON BREHM-STECHER: *Iowa State University, Ames, IA, USA*
CINDY NAKATSU: *Purdue University, West Lafayette, IN, USA*
AHMED YOUSEF: *The Ohio State University, Columbus, OH, USA*

Cultural techniques have served as the backbone of modern microbiology from its inception until recent history. Cultural techniques provide researchers and outbreak investigators with the isolates needed for further study and characterization. Unfortunately, microbiologists have long struggled with an inability to culture the microbial majority, a conundrum known widely as "The Great Plate Count Anomaly". This limitation has hampered progress in our understanding of microbial ecology, behavior and infectivity. Recent advances in culture-independent methods allow unprecedented access to key information about microorganisms in the environment, in our food or in (and on) our bodies. In their excitement over the promise of these new methods, some microbiologists have gone so far as to declare that microbial culture is dead.

The purpose of this short symposium is to examine recent advances in our understanding or application of cultural techniques that highlight the continued and unique value of microbial culture. Although the topics are sourced from various microbiological disciplines, these examples contain valuable lessons for microbiologists working at all points across the farm-to-fork-to-physician continuum.

Topics include the recent and discovery of a fundamental pitfall in growth medium preparation, formulation of novel food-based media for enhanced recovery of spoilage organisms and a review of recent innovations in microbial culture techniques. We expect this session will promote a lively cross-disciplinary conversation about the continued utility and future of cultural techniques.

S17 Wash Water Management for Post-harvest Washing of Fresh-cut Produce

TONG-JEN FU: *U.S. Food and Drug Administration, Division of Food Processing Science and Technology, Bedford Park, IL, USA*
ELLIOT RYSER: *Michigan State University, East Lansing, MI, USA*
JIM BRENNAN: *SmartWash Solutions, LLC, Salinas, CA, USA*

Fresh produce is attributed to a significant number of foodborne illnesses in the U.S. Contamination that occurred on-farm can persist and spread during postharvest handling. Past outbreak investigations have suggested that postharvest washing could be the point where contamination spreads. Postharvest washing is also the most practical point of control. The use of sufficient antimicrobial chemicals in wash water is critical to minimizing the potential of pathogen cross-contamination. While a great deal of efforts have been made by the fresh-cut industry to develop and implement wash water management programs, the effectiveness of these measures remains to be determined.

Industry and government guidelines have recommended that wash water antimicrobials be monitored to maintain sanitary conditions. Specific performance criteria for certain types of commodities have been recommended. With the enactment of the Food Safety Modernization Act (FSMA), the fresh produce industry will need to validate the efficacy of its washing process at preventing microbial cross-contamination. However, questions remain regarding how a produce washing process should be validated.

This symposium will highlight current advances and challenges associated with wash water management at produce processing facilities. Speakers will discuss the risk of pathogen cross-contamination during washing of fresh-cut produce. Innovations in wash water management practices implemented in commercial packing facilities will be presented. Validation strategies for produce washing will be discussed. A case study on how a process validation could be performed for flume washing of cut-lettuce will also be presented.

S18 Complexity in Managing Risk from Pathogens in the Fresh Produce Chain: How Can Risk Assessment Help?

ROY BETTS: *Campden BRI, Gloucestershire, United Kingdom, United Kingdom*
YUHUAN CHEN: *U.S. Food and Drug Administration, College Park, MD, USA*
MICHELLE D. DANYLUK: *University of Florida, Lake Alfred, FL, USA*

Managing risks from pathogens in the fresh produce chain (on farm, processing, and retail) continues to represent a complex food safety challenge. Pathogen contamination of produce, including products as diverse as leafy greens and tree nuts, may be influenced by complex environmental factors and food safety practices during production, harvesting, processing, and handling subsequent to harvest. On the one hand, microbial risk assessment (MRA) as a tool may be well suited to address complex food safety issues in which quantitative risk models can integrate a multitude of data and information on the pathogen, the food, and the consumer to predict effectiveness of prevention and control practices. On the other hand, to stakeholders (such as industry stakeholders), the information on MRAs that, currently, exists in the public domain is sometimes highly theoretical and very generic; application to each industry sector can be difficult, even though regulatory agencies increasingly use risk assessment to inform decision making, such as in proposing a new standard and drafting guidance to industry. The purpose of this session is to provide a public-private view with speakers from industry, academia, and government. Presentations will include findings of a recent ILSI Europe expert group on industrial MRA in fresh produce, ongoing produce risk assessment designed to inform regulatory decision, and an academic perspective on the latest data collection and risk assessment efforts. This session will aim to provide examples for complex, as well as easy-to-follow and practical risk assessments specific to fresh produce, to inform the audience on the implementation of risk assessment strategies.

S19 How Does GFSI Audit Criteria for Sanitation, Hygiene, and Environmental Sampling Compare to FSMA Requirements?

ROBERT PREVENDAR: *NSF International, Ann Arbor, MI, USA*
LEANN CHUBOFF: *Safe Quality Food Institute, Chicago, IL, USA*
PAUL HALL: *Flying Food Group, Lakeland, FL, USA*

There have been numerous discussions and comparisons between US FDA regulated food processing companies that have been under a GFSI program, for some time, and FSMA regulations using the HARPC scheme. This three speaker symposium's objective is to assess the impact of the phased rollout of FSMA, in the U.S. by FDA, with plants already certified under a GFSI scheme. The session will include experienced experts from GFSI certifying bodies and the industry. Participants will share experiences about the first year of FSMA implementation, with emphasis on Supply Chain Preventive Controls. Differences between GFSI schemes and FSMA, based on plant size, will be discussed. Also, time permitting, the presenters will provide feedback and advice for improvement in an open forum.

S20 A Risk-based Approach to Microbiological Performance Criteria for Addressing Pathogens in Meat and Poultry

MICHAEL WILLIAMS: *U.S. Department of Agriculture-FSIS, Washington, DC, USA*
BARBARA KOWALCYK: *RTI International, Research Triangle Park, NC, USA*
CRAIG HEDBERG: *University of Minnesota, School of Public Health, Minneapolis, MN, USA*

The U.S. Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) establishes microbiological performance standards for industry to achieve national food safety goals. USDA-FSIS also conducts regular testing of food products to and verify an establishment's process and controls meet these standards. Microbiological standards are product-specific and based on qualitative results (e.g., presence or absence of *Salmonella* spp.). In 2015, USDA-FSIS proposed new pathogen reduction performance standards for *Salmonella* and *Campylobacter* in chicken parts and comminuted poultry based on results of a quantitative risk assessment designed to achieve national food safety goals (Healthy People 2020) for reducing *Salmonella*- and *Campylobacter*-related illness in those products under its regulatory purview. USDA-FSIS verifies an establishment meets pathogen reduction performance standards through federal qualitative microbiological analysis of product samples.

This symposium discusses prevalence-based microbiological performance standards as well as semi-quantitative criterion developed to control *Salmonella* contamination in meat and poultry and their impact in public health. It will present FSIS's quantitative risk assessment used to inform the development of the current microbiological performance standards. This symposium will also explore two risk assessments conducted, using both public and private sector data, to evaluate the public health impact of industry adoption of a semi-quantitative operational microbiological performance criterion for *Salmonella*.

S21 Do Not Stumble Over a Process Deviation: Regain Control with Predictive Microbial Modeling

TIMOTHY MOHR: *Science Staff/OPHS/FSIS/USDA, Salem, OR, USA*
DONALD W. SCHAFFNER: *Rutgers University, New Brunswick, NJ, USA*
HARSHAVARDHAN THIPPAREDDI: *University of Georgia, Athens, GA, USA*

SIS will be discussing their revised Compliance Guidelines for Appendix A (Lethality) and Appendix B (Stabilization) that will have new information on using pathogen modeling to evaluate heating and cooling deviations. Presenters from USDA, Academia, and Industry will discuss through case studies how to evaluate type I heating deviations (i.e., failure to meet critical limit of cooking CCP) and type II heating deviations (i.e., slow heating come-up time) in cooked/heat-treated meat and poultry products, and cooling deviations in cooked/heat-treated meat and poultry products. Specifically, the presentation for each type of process deviation will cover the following topics: FSIS requirements and/or policy for lethality or stabilization; the biological hazards associated with the process deviation; available predictive microbial models; performance of models; the appropriate approach and important considerations when using a model from a FSIS perspective; key information needed to conduct successive predictive microbial modeling; the level of pathogen growth or lethality that would be regulatory noncompliance and a public health concern; and actual process deviation scenarios.

S22 Defining, Capturing, and Assessing the Vulnerability of the Food Supply to Economically Motivated Adulteration (EMA) and Food Fraud

KAREN EVERSTINE: *USP, Rockville, MD, USA*
QUINCY LISSAUR: *SSAFE, London, United Kingdom, United Kingdom*
BRIAN HAWKINS: *Battelle, Columbus, OH, USA*

Economically motivated adulteration (EMA) is a threat to the integrity of the food supply chain, with potential impacts ranging from loss of consumer trust, to brand reputation damage, to food safety. With the increasingly global and dynamic nature of today's food supply chain, an improved understanding of EMA and maturation of approaches to assess and combat EMA are critical to modern food safety and defense. Pulling from a combination of industry and academic experience, this session focuses on providing a clear understanding of what EMA is and is not (e.g., how does EMA differ from food fraud?); discussing how EMA incidents can be captured and cataloged, in order to improve awareness of events; presenting survey results that capture industry perspectives on factors affecting EMA; and discussion of approaches to assess EMA vulnerabilities, including validation results using historical data.

S23 How to Exploit Omics Data on Pathogen Behavior in Microbiological Risk Assessment: An Update on the Current Research

KALLIOPI RANTSIOU: *University of Turin-DISAFA, Turin, Italy, Italy*
HEIDY DEN BESTEN: *Wageningen University, Wageningen, Netherlands, Netherlands*
TREVOR PHISTER: *PepsiCo, Leicester, United Kingdom, United Kingdom*

Following the technological advancements in the field of the nucleic acids sequencing and the possibility to obtain a large number of sequences (millions) from the microorganisms present in a single sample without the need for their cultivation, new opportunities have become available in terms of data production and exploitation in the field of microbiological risk assessment (MRA). More specifically, the behavior of foodborne pathogens, deciphered with transcriptomic, proteomic, and/or metabolomics techniques, during the whole food chain and in response to specific stresses, can now be studied. The current challenge scientists are facing is the integration of such data into risk assessment schemes.

In 2016, at the IAFP European Symposium in Athens, the workshop, "Next Generation MRA (Microbiological Risk Assessment) - Integration of Omics Data into Assessment" was co-organized by ILSI Europe, IAFP, and ICFMH. During the workshop, four breakout groups (epidemiology, metagenomics, exposure assessment, and hazard characterization) brain-stormed and produced a common strategy to go beyond the current knowledge. In this symposium we will present the main outcomes of the workshop, including the points of view of the academia and the industry, and we will advance the discussion related to how to best use omics data in MRA.

S24 Battling Bad Bugs: Biological Approaches to Control Pathogens

WOJCIECH JANISIEWICZ: *U.S. Department of Agriculture–ARS, Kearneysville, WV, USA*
MINDY BRASHEARS: *Texas Tech University, Lubbock, TX, USA*
SAM ALCAINE: *Cornell University, Ithaca, NY, USA*

The food industry often relies on physical and chemical interventions to control foodborne pathogens. This session is intended to discuss biological approaches to control foodborne pathogens throughout the food continuum. Topics to be covered include the use of (i) biocontrol of *Listeria monocytogenes* and *Salmonella enterica* Serovar Poona on fresh-cut apples with naturally occurring bacterial and yeast antagonists, (ii) probiotics to reduce the load of foodborne pathogens that enter the human food chain and pathogen populations on food and in food processing environments and (iii) bacteriophage to combat bacterial foodborne pathogens. This session will, also, include discussions on consumer perception and acceptance of these biological approaches to control foodborne pathogens.

S25 Non-thermal Plasma Technology for Improving Food Safety and Quality

ALEXANDER FRIDMAN: *Drexel University, Philadelphia, PA, USA*
ROGER RUAN: *University of Minnesota, Saint Paul, MN, USA*
BRENDAN NIEMIRA: *U.S. Department of Agriculture-ARS, Wyndmoor, PA, USA*

There are increasing interests in nonthermal food processing because of demands for safe, nutritious, and high quality food products. Nonthermal plasma (NTP) or cold plasma is a promising emerging technology for food processing and preservation, particularly for nonthermal pasteurization and disinfection of liquid and solid foods, as well as sanitation of process water and equipment. The technology may also be used in waste and odor management in the food industry. This symposium is among the first to discuss applications of cold plasma technology in the food industry. The symposium will provide an overview of current research and engage in discussion on future developments in NTP technology. The speakers will be leading scientists and engineers from academia, national research institutes, and industry. A comprehensive treatment of the topic spans from fundamentals of NTP science, engineering and technology developments, effectiveness and constraints in applying NTP to improve food safety and quality, mechanisms of cold plasma microorganism inactivation, chemical response of food components and the environment, case studies on nonthermal pasteurization and disinfection of foods, applications in waste and odor management, development of NTP processes and equipment, to economic and environmental

assessment. The primary objectives of the symposium are to foster in-depth discussions on the technical and economic feasibility of the technology, identify the gaps and opportunities for future research in order to move the technology to the commercial sectors, and assist federal agencies in developing research investment priorities and regulatory guidelines.

S26 Let's Get Active!

CYNTHIA EBNER: *Sealed Air Corporation, Duncan, SC, USA*
JOE DUNN: *Performance Packaging of Nevada, Daytona Beach, FL, USA*
S. BALAMURUGAN: *Agriculture & Agri-Food Canada, Guelph, ON, Canada, Canada*

If you thought food packaging was just simple "plastic", have we got news for you! Modern food packaging products can provide an active function beyond inert passive containment and protection of food; active packaging is designed to do far more. Active packaging incorporates components that are released into the packaged food or its surrounding environment, including material intended to maintain or improve the safety, shelf life, and quality of the packaged food. Alternatively, the active packaging can absorb substances from the packaged food or its surrounding environment, including those that may negatively impact the quality or safety of the food product.

This session will discuss active packaging, in general and several specific technologies, including packages with the ability to scavenge malodors from packaged food and other active packages that can help maintain food quality and safety by scavenging excess oxygen inside the package. Active packaging can, also, help improve food safety and shelf life by incorporating antimicrobial agents that can control unwanted microorganisms on the packaged food. An exciting new approach, that will be presented in this session, is use of bacteriophages as antimicrobial agents in active packaging to enhance microbial safety of foods.

S27 Biological Soil Amendments of Animal Origin and the Food Safety Modernization Act: Challenges and Opportunities Going Forward

DAVID INGRAM: *U.S. Food and Drug Administration, College Park, MD, USA*
ALDA PIRES: *University of California-Davis, Department of Population Health & Reproduction, Davis, CA, USA*
ELISABETTA LAMBERTINI: *RTI International, Rockville, MD, USA*

Use of biological soil amendments of animal origin (BSA of AO) are important to growers managing fresh fruit and vegetable production systems; particularly organic growers who are prohibited from using synthetic fertilizers. Pathogen survival in amended soils and transfer to fresh produce is a food safety concern addressed in the FDA Produce Rule. Growers, extension specialists, and fresh produce food safety trainers are seeking additional information about the current regulatory thinking on BSA of AO, while researchers would like to have a better understanding of the scope of use of BSA in current production practices. This session will address both of these issues and open a dialog on this important topic.

S28 The Produce Safety Alliance: From Education and Training to Implementation and Beyond

DONNA PAHL: *Cornell University, Riverside, CA, USA*
ELIZABETH NEWBOLD: *University of Vermont, Bennington, VT, USA*
JAMES RUSHING: *JIFSAN-University of Maryland, College Park, MD, USA*

Since 2010, the Produce Safety Alliance (PSA) has been developing a standardized produce safety training curriculum to prepare fresh produce growers to meet the regulatory requirements in the U.S. Food and Drug Administration's (FDA) Food Safety Modernization Act (FSMA) Produce Safety Rule. Through a five-year development process that included an educational materials conference, eight focus groups with produce growers, ten public working committees, and coordination with the FDA Division of Produce Safety to align the curriculum content with the regulation, a seven module Grower Training Curriculum was developed. This symposium will begin by sharing key milestones and current training progress. Providing training and technical assistance to produce growers, packers, and industry members is a critical component to achieving successful implementation of the new produce safety standards. Starting in September 2016, the PSA launched both Train-the-Trainer and Grower Training Courses to address this need. During the session, presenters will discuss current regional and international education and outreach efforts, as well as challenges in providing effective training to growers. The symposium will, also, offer insight into opportunities, for Extension personnel and other educators, to fill these knowledge gaps and training challenges through the development of new and innovative educational resources. Lastly, it is important to not lose sight of the many growers who may be exempt or not covered by the regulation, but who may be subject to buyer requirements for produce safety practices. Although there are barriers to reaching small and limited resource growers, there are ways to motivate and support them in the implementation of produce safety practices, regardless of whether that pressure is regulatory or market driven. The session will conclude by discussing future opportunities for partnerships and educational initiatives, across the country and internationally, to make training accessible to all growers.

S29 After 20 Years of Seafood HACCP, is Our Food Safer?

LISA WEDDIG: *National Fisheries Institute, McLean, VA, USA*
STEVEN BLOODGOOD: *U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA*
ERIN BURDETTE: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
JOHN JACOBS: *NOAA, Oxford, MD, USA*
JONATHAN DEEDS: *U.S. Food and Drug Administration–CFSAN, College Park, MD, USA*
STEVE OTWELL: *University of Florida, Gainesville, FL, USA*

December 2017 marks the 20th anniversary of the implementation of the U.S. Food & Drug Administration's seafood HACCP regulation (procedures for safe and sanitary processing and importing of fish and fishery products). This symposium will look back at the conditions that prompted this historic change in food safety regulation and assess the impact it has had on the seafood industry, food safety overall, and implementation of subsequent HACCP rules. Although foodborne outbreaks due to seafood have significantly decreased in the U.S. since 2005, Vibrio illnesses and harmful algal blooms (HABs) appear to be increasing and, geographically, expanding. Are these increases the result of climate change, and can HACCP evolve to meet these challenges? Does the seafood industry need new detection and control methods to supplement HACCP? Whether accidental or deliberate, species substitution continues to be a problem, resulting in economic fraud and potentially endangering the health and welfare of seafood consumers through introduction of toxic species and/or allergens. As our global seafood supply progressively relies on aquaculture, antibiotic drug residues can pose long-term health risks, if improperly used. Now that the Food Safety and Modernization Act (FSMA) is being phased in, how will it affect the seafood industry? HACCP guidance and interpretations are changing to address these and other emerging issues. In this symposium, we will hear from industry representatives, regulators, and others about the current state of seafood safety. We will celebrate the successful marriage of HACCP and seafood; but,

we will, also, take a critical look at hazards and problems that may require at least partial reliance on additional methods or tools to enhance seafood safety in the future.

S30 Strategic Intervention Design: A Pragmatic Approach to Validation

GARY ACUFF: *Texas A&M University, College Station, TX, USA*
JAMES DICKSON: *Iowa State University Food Microbiology Group, Ames, IA, USA*
PABLO ALVAREZ: *Novolyze Inc., Cambridge, MA, USA*

After the recent release of FSMA final rules and industry guidance, including Preventive Controls for Human Food and Foreign Supplier Verification Program, many food producers are struggling with the challenge of implementation due to the variety and complexity of processes requiring validation. Validation can be a complex process and a number of different parameters and techniques should to be considered in order to assure that process interventions designed to control pathogenic bacteria are functioning correctly and achieving desired goals. Proper experimental design, analysis, and interpretation are required to provide data that will stand up the regulatory scrutiny and customers needs.

The utilization of surrogates to validate a food process is not a new concept; however, the choice of an optimal surrogate can only be made after taking into account the process to validate, the food matrix, and the specific pathogen of interest. The aims of this symposium are to provide a pragmatic approach to process validation, answers to common questions that food processors have, and guidance for success.

S31 Development of Microbiological Criteria as Indicators of Process Control or Insanitary Conditions: A Summary of the Report Prepared for the United States Department of Defense by the NACMF

STEVE INGHAM: *Wisconsin Department of Agriculture, Trade and Consumer Protection, Madison, WI, USA*
KATHLEEN GLASS: *University of Wisconsin-Madison, Madison, WI, USA*
MARGERY HANFORD: *US Army Medical Research Institute of Chemical Defense (USAMRICD), Aberdeen Proving Ground, MD, USA*

The Department of Defense (DOD) requested that the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) provide guidance on the development of microbiological criteria for indicators of process control or insanitary conditions that could be used by DOD in its evaluation of food suppliers, world-wide. While recognizing the importance of multiple food supplier programs that should be implemented and evaluated, e.g., statistical process control and sanitation effectiveness monitoring, NACMCF recognized that in many situations DOD must evaluate supplier suitability using standardized microbiological sampling and testing programs that could provide an indication of poor process control or insanitary conditions. For 47 food categories commonly procured by DOD, the NACMCF report presents generic process flow diagrams with process steps identified where microbial contamination, microbial growth, pathogen reduction, and microbiological sampling may occur. For each food category, environmental monitoring program target microorganisms are recommended, along with microbiological limits recommended for use under routine and non-routine circumstances. The report recommends actions to be taken, if these microbiological limits are exceeded. The report, also, provides valuable insight into establishment of microbiological criteria for use in a statistical process control regime. The NACMCF sub-committee preparing this report was Co-Chaired by Robert (Skip) Seward and Jeff Kornacki and the report was recently published on line at *www.fsis.usda.gov/wps/wcm/connect/2ea3f473-cd12-4333-a28e-b2385454c967/NACMCF-Report-Process-Control-061015.pdf?MOD=AJPERES*

S32 What Can We Do with 10,000 Genomes That Couldn't be Done with 100?

YAN LUO: *U.S. Food and Drug Administration, College Park, MD, USA*
DAVID GALLY: *University of Edinburgh, Edinburgh, United Kingdom, United Kingdom*
LAWRENCE GOODRIDGE: *McGill University, Ste-Anne-de-Bellevue, QC, Canada, Canada*

In less than 15 years, genome sequencing and analysis has progressed from a luxury available to only the few to one widely used across academic, government, and industry laboratories. What started as the sequencing of model organisms grew into comparative genomics of a handful of related species; and today there are over 10,000 publicly available genomes for a number of species. Interestingly, many of these are foodborne pathogens, including *Salmonella enterica*, *Escherichia coli*, and *Listeria monocytogenes*. Genome data is increasingly being associated with available metadata, as well. These advances raise two questions that presenters will be asked to clarify:

1. What biological insights can we gain from tens of thousands of genomes that were not possible only five to six years ago?
2. How can our community leverage these foodborne pathogen-biased datasets to lead new developments, such as the linking genotype to phenotype?

S33 What Can Complete Closed Microbial Genomes Provide to Food Safety?

JAMES BONO: *USDA ARS U.S. Meat Animal Research Center, Clay Center, NE, USA*
DANIEL HURLEY: *University College Dublin, Dublin, Ireland, Ireland*
JAIME MARTINEZ-URTAZA: *University of Bath, Bath, United Kingdom, United Kingdom*

We are now able to characterize completely closed microbial genomes using the third-generation, single molecule, real-time DNA sequencing on the Pacific Bioscience (PacBio) Sequencer. Currently, it is frequently challenging to correctly assemble bacterial genomes and plasmids from short reads, due to the frequency of repetitive regions. With long read technology, we can assemble, correctly, through large mobile elements such as phages, plasmids, insertion sequences, and pathogenicity islands. These enhanced sequencing capabilities provide a comprehensive view of genetic composition that allows the generation of:

1. high quality reference genomes for source tracking during a foodborne outbreak investigation,
2. understanding long-term evolution of foodborne pathogens,
3. new insights in drug resistance and transmission of mobile elements carrying antimicrobial resistance markers, and 4) information about the contribution of DNA modification on pathogenesis.

The benefits of using closed genomes in routine surveillance will be discussed. In this session, we are going to present examples where the use of closed genomes allowed answering diverse questions, which could not have been achieved using draft genomes generated by short reads technology. The use of closed genomes will allow the regulatory agencies and the food industry to gain a greater understanding of the genetic compositions of virulence genes, as well as antimicrobial resistance genes presence in these isolates; both targets being equally important when tracking and treating illnesses caused by these pathogenic organisms. Further, the availability of a fully closed reference genomes increases accuracy of clustering, which is

needed to facilitate outbreak cluster detection and source tracking and to provide deeper insights into structural variations, which is critically important for public health.

S34 Managing Risk in a Zero Tolerance World

DON ZINK: *IEH Laboratories & Consulting Group, Herndon, VA, USA*
ROBERT BUCHANAN: *University of Maryland, College Park, MD, USA*
LUCA COCOLIN: *University of Torino-DISAFA, Grugliasco, Italy, Italy*
ROY BETTS: *Campden BRI, Gloucestershire, United Kingdom, United Kingdom*
TIMOTHY JACKSON: *Nestle USA, North America, Glendale, CA, USA*
WILLIAM HALLMAN: *Rutgers University, New Brunswick, NJ, USA*

It is accepted that there is risk in everything and the discipline of risk assessment focuses on minimizing risk to an acceptable level. This seems to be in conflict with the concept of zero tolerance, which is not completely codified in regulation; and yet, does seem to exist in actual food industry practice. Does the current interpretation of FSMA rules and regulatory application of new technologies in outbreak investigation drive the expectation of a zero-risk environment? What should risk assessment and food safety management look like in this changing landscape? How can risk be managed to allow suitable options for consumers and food manufacturers? The perception and understanding of risk necessitates a conversation between regulators, consumers, and the food industry.

S35 Novel and Not-so-Novel Cleaning and Sanitizing Methods

JEFFREY KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*
JOHN MERENICK: *Sargento, Plymouth, WI, USA*
ROBIN PETERSON: *Micreos, Atlanta, GA, USA*
TBD TBD: *TBD, TBD, CA, USA*
NATHAN MIRDAMADI: *Commercial Food Sanitation, Aliquppa, PA, USA*
EVAN ROSEN: *PacMoore, Hammond, IN, USA*

Low moisture foods are often associated with allergen recalls and there is an increased number of recalls associated with microbial contamination of ready-to-eat low moisture foods. While many of the allergen recalls are labeling related, using verified cleaning methods to prevent cross-contamination continues to be a preoccupation for the industry. In addition, following the publication of warning letters and recalls associated with the presence of *Listeria monocytogenes* in low moisture foods, many are challenged to find effective remediation methods. During this symposium, we will discuss and provide examples of novel and not-so-novel cleaning methods that industry can use to prevent chemical and microbial cross contamination. A number of technologies exist that provide alternative approaches; these include pathogen phages, sterilant gases, CO2 entrained alcohol, heat (hot oil, dry, and wet heat), dry ice blasting, and others. There is also confusion about the need to verify or validate, or both, cleaning and sanitizing methods in the FSMA-era and to reconcile what needs to be done to meet the different audit schemes. We intend to provide examples and solutions that will generate discussions with the attendees and introduce new ideas.

S36 Getting to the Reality of Implementation: Produce Safety Rule Water Quality Requirements

MICHELLE D. DANYLUK: *University of Florida, Lake Alfred, FL, USA*
FAITH CRITZER: *University of Tennessee, Department of Food Science, Knoxville, TN, USA*
TREVOR SUSLOW: *University of California-Davis, Davis, CA, USA*

The Food Safety Modernization Act's regulations were developed to proactively address food safety. Impacts of these regulations will be far-reaching and have already changed food safety management within operations. Some of the rules, such as those addressing produce safety, provide requirements to an industry where federal food safety regulations have, previously, been absent. Therefore, the question of compliance and implementation is germane. The area of "agricultural water" is one of the most vigorously debated parts of the Produce Safety Rule. Some anxiety and obstacles have been triggered by the expectation that "it must be safe and of adequate sanitary quality for its intended use." Evaluation of untreated agricultural water quality must be done through establishment of a microbial water quality profile, through representative samples by an indicator of water quality, specified as generic *Escherichia coli*; but allowance for qualified alternatives is available. Finding labs accredited in U.S. EPA method 1603 or defining equivalent methods that could be used are outstanding shared barriers to implementation across many farms with "covered" produce. Interestingly, many qualified exemption farms are already being required to implement this Produce Rule ag-water testing. When the required microbial water quality profile is not meet, treatment of water with an EPA labeled product is one of the allowed corrective measures. Many are having difficulty identifying what their treatment options are and determining what treatment system is appropriate for their operation. FDA guidance documents can provide further assistance for defining best practices, but there will be remaining obstacles. This symposium will provide the critical hurdles and robust discussion for moving us towards required industry compliance under the Produce Safety Rule, in the areas of establishing microbial water quality profiles, corrective measures, and remaining areas for consideration, if we are to achieve the required compliance under this rule.

S37 Pro- and/or Pre-biotics as Bio-remedies and Foodborne Infection Preventives

DEBABRATA BISWAS: *University of Maryland, College Park, MD, USA*
SEONG-HO LEE: *University of Maryland, College Park, MD, USA*
SHAIK RAHAMAN: *Nutrition and Food Science, University of Maryland, College Park, MD, USA*

As a major source of microbes and their numerous beneficial effects, the gut microflora/microbiome is intimately linked to human health, immunity, and disease. The key intestinal microbial byproducts, commonly known as secondary metabolites (SM), are crucial to the maintenance of a balanced gut ecosystem and healthy gut microbial community. Moreover, SM play multiple critical roles in host defense and immunity, including anti-cancer, anti-inflammation, and anti-oxidant activities, as well as outcompetition of enteric bacterial pathogens. In this symposium, we aim to highlight the importance of probiotics, prebiotics, their combination (symbiotic), and SM in a balanced gut ecosystem, immune health, and their interactions with enteric bacterial pathogens and other diseases. Finally, we will advance potential applications of the bio-remedies, with regards to controlling diseases and improving human health.

S38 Moving toward the Safe Use of Recycled Water for Crop Irrigation: A Sustainable Solution in an Era of Climate Variability?

AMY SAPKOTA: *Maryland Institute for Applied Environmental Health, University of Maryland, School of Public Health, College Park, MD, USA*
CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*
CLIVE LIPCHIN: *Arava Institute for Environmental Studies, Ketura, Israel, Israel*

Our changing climate, escalating water demands from non-agricultural sectors, and depletion of groundwater sources by agricultural use are immediate challenges that call for the urgent need to explore and adopt safe, alternative irrigation strategies to sustain food production across the U.S. As a result, water reuse and the exploration of nontraditional irrigation sources have become national priorities with regard to agricultural water security and the sustainable production of our food supply. At the same time, the recent Food Safety Modernization Act is shifting the focus of food safety from responding to contamination to preventing it. This emphasis towards the prevention of foodborne illnesses places great responsibility on agricultural producers, who must meet stricter guidelines with regard to the quality of irrigation water used on food crops. Hence, at this critical juncture in food production, sustainable on-farm solutions are needed to enable agricultural producers to conserve groundwater and adopt safe and innovative approaches to irrigation. The development of strategies to enable the microbiological, chemical, and physical safety of recycled water will enable food production to thrive despite our erratic climate. However, successful implementation of water reuse strategies will depend not only upon scientific advances in technology to assure the safety of recycled water, but also upon acceptance and support from growers, consumers, and regulatory agencies. This symposium will bring together speakers who will describe a complex global picture of the potential for recycled water to become a more commonly accepted and used source for irrigation of crops. As we move into a time of increasing water scarcity and inconsistency, a shift in water use strategies will be essential to sustain food production in arable land across the nation.

S39 Water, Water, Everywhere: The Effects of Flooding on the Microbial Safety of Fresh Produce

PETER BERGHOLZ: *North Dakota State University, Fargo, ND, USA*
LAURA STRAWN: *Virginia Tech - Eastern Shore AREC, Painter, VA, USA*
MELANIE IVEY: *The Ohio State University, Wooster, OH, USA*

Natural disasters, such as flooding, contribute to a variety of physical, chemical, and/or microbiological hazards to crop systems and can cause major public health risks through fresh produce. Human pathogens such as norovirus, *Salmonella*, *Campylobacter*, and *Escherichia coli* O157:H7, can be introduced into fresh produce in the field during flood events through exposure to raw sewage from farm animals, such as chickens, swine, and goats, river or pond surface waters, and agricultural runoff. Researchers have shown that the microbial hazard, following a flood event, is not only from fruits and vegetables directly in contact with flood water, but, also, from the edible portions not in contact with flood water. Three presentations will flow, geographically, from New England, to the eastern shore of Virginia, and then to the southern state of Louisiana. The first presenter will discuss his research on mapping microbial contamination of two produce farms after catastrophic flooding caused by Hurricane Irene. He collected prevalence and genetic data. The ecology of pathogens associated with surface water under normal and flood conditions will be discussed. In the second presentation, the speaaker will discuss her exciting data looking at the effects of flooding on the prevalence and diversity of *Salmonella* on the eastern shore of Virginia, over time. The third presenter will talk about the survival of fecal indicators and the presence/absence of foodborne pathogens on cantaloupes after flooding in Louisiana.

S40 Ensuring Food Safety through the Product Development Lifecycle: Successes and Pitfalls

KATHRYN MCCANN: *The Kellogg Company, Battle Creek, MI, USA*
JOSEPH MEYER: *The Kraft Heinz Company, Glenview, IL, USA*
KATHLEEN GLASS: *University of Wisconsin-Madison, Madison, WI, USA*

A key focus for food safety professionals in developing new products is to protect public health by designing foods that are safe, palatable, and nutritious throughout a product's lifecycle. In a rapidly changing global market place where speed to market may dictate success or failure of a new product concept, companies that take too long to commercialize their products may fail to capitalize on a narrow window of opportunity. One of the most difficult challenges that food safety professionals face, however, is how to be more flexible and be able to adapt in a dynamic business climate. How should food safety professionals respond to market needs and changes in the business environment, capitalize on opportunities, and navigate around risks and regulatory challenges? Learn approaches on how to create a balance in innovation, business opportunity, while ensuring food safety.

S41 Clean Label Technologies for Safety of Processed Meat and Poultry Products: Scientific Support of Efficacy

KATHLEEN GLASS: *Food Research Institute, University of Wisconsin-Madison, Madison, WI, USA*
JAMES DICKSON: *Iowa State University, Ames, IA, USA*
AARON ASMUS: *Hormel Foods, Austin, MN, USA*

Recently, significant advancements have occurred in the area of clean label food safety technologies, including identification of ingredients not previously believed to play a role in food safety. This symposium addresses multiple program areas, including safety and microbial quality of meat and poultry, food toxicology, and food regulations, making it of interest to attendees from large and small meat processors from within and outside the U.S., as well as food processors or retailers that utilize clean label meat and poultry products. Research surrounding food safety technologies to enable clean labels on processed meat and poultry products has been ongoing for many years. However, peer reviewed publications in the last two years have clearly illustrated the antimicrobial efficacies of clean label alternatives relative to traditional ingredients, such as celery powder and sodium nitrite, or cherry powder and sodium ascorbate.

S42 Mechanisms of Hypervirulence in Selected Foodborne Pathogens

JOHN MAUER: *University of Georgia, Athens, GA, USA*
QIJING ZHANG: *Iowa State University, Ames, IA, USA*
VICTORIA JEFFERS: *Indiana University, Bloomington, IN, USA*

Increases in the number of outbreaks and severity of infection resulting in high mortality has led to the recognition that not all foodborne pathogens are equally virulent. Hypervirulence is defined as a state in which a pathogen exhibits heightened abilities to cause disease, whether by increased invasiveness, enhanced ability to evade host defenses or through other mechanisms. In this session, a panel of experts will discuss the genetic, phenotypic, physiological and other bases of hypervirulent behavior in various foodborne pathogens, including *Salmonella*, *Campylobacter jejuni* and Toxoplasma gondii.

S43 The Crossroad between Global Trade and Food Safety: Focus on Viruses and Parasites

ROSA PINTO: *University of Barcelona, Barcelona, Spain, Spain*
RICHARD BRADBURY: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
DORILIZ DE LEON: *U.S. Food and Drug Administration–CFSAN, College Park, MD, USA*

Foodborne viruses and parasites have become increasingly associated with cases of illness from international trade. More specifically Hepatitis A virus (HAV) has been implicated in outbreaks, both in the U.S. and Europe, associated with several imported products, including sundried tomatoes, red currents, pomegranate seeds, scallops, and frozen berries. Parasites, such as Cyclospora cayetanensis, have been associated with large outbreaks in the U.S. and Canada; linked to consumption of imported fresh produce. This symposium will focus on the issues revolving around international trade and the spread of foodborne parasites and HAV from different food commodities, as well as improvements in compliance to prevent such outbreaks. Featured speakers are Dr. Richard Bradbury, speaking on parasites; Dr. Rosa Pinto, highlighting the HAV epidemiological traits and food safety concerns associated with the foodborne transmission of this virus. Lastly, Dr. Doriliz de Leon will speak about regulatory compliance and consumer protection.

S44 Modeling Pathogens in Low-water Activity Foods: What, How, and How to Use It

SARAH CAHILL: *Food and Agriculture Organization of the United Nations, Rome, Italy, Italy*
SOFIA SANTILLANA FARAKOS: *U.S. Food and Drug Administration, College Park, MD, USA*
LINDA J. HARRIS: *University of California-Davis, Davis, CA, USA*

Managing microbial hazards in low water activity foods continues to pose a challenge to the food industry. Models in food microbiology can provide useful information for setting performance standards and food safety objectives. The first step is to identify the question that needs to be answered. A modeling approach is, then, selected to quantitatively describe the process. This symposium will provide an introduction to the risks and how to rank microbial hazards in low water activity foods. This will be followed by how to develop and validate models and their use in risk assessment. Finally, the application of models and risk assessment, in an industry setting, and the importance of process validation will be discussed.

S45 Can Old Processes Satisfy New Rules? Pathogen Reduction in Legacy Processes for Low-moisture Foods

NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
BRADLEY MARKS: *Michigan State University, East Lansing, MI, USA*
JEYAMKONDAN SUBBIAH: *University of Nebraska-Lincoln, Lincoln, NE, USA*

A wide portion of the low-moisture food processing industry is comprised of legacy thermal processes (e.g., roasting, toasting, baking, drying) that were historically designed, implemented, and operated to achieve food quality goals, rather than food safety outcomes. However, under the FSMA Preventive Controls rules, such processes must be validated to achieve appropriate pathogen (*Salmonella*) reduction, regardless of their historical track record, unless additional unit operations are specifically implemented for pasteurization. Given the enhanced thermal resistance of *Salmonella* in low-moisture systems, the dynamic and complex heat and mass transfer phenomena occurring during these processes, and the difficulty in real-time monitoring of critical variables, validation of such processes is not a trivial task. Additionally, particularly for small and medium-sized processors, investment in stand-alone pasteurization technologies might not be feasible, making validation and/or modification of existing processes essential from both a regulatory and economic perspective. Therefore, this symposium will present analyses of three possible pathways for processors to meet this challenge:

1. Using a systems (hurdle) approach to ensuring pathogen reduction in low moisture foods;
2. Modifying existing processes that might otherwise be insufficient as traditionally operated, in order to enhance pathogen reduction; and
3. Choosing among various options for stand-alone pasteurization technologies. The speakers will present an overview of these approaches, including the regulatory expectations, recent research results across multiple products and processes, and a critical review of the state-of-technology for pasteurization options, from the perspective of multi-criteria decision analysis.

S46 Cross Pollination of *Listeria* Learnings across the Industry

JOHN ALLAN: *International Dairy Foods Association, Alexandria, VA, USA*
MATTHEW RANIERI: *Acme Smoked Fish Corporation, Brooklyn, NY, USA*
JOSEPH STOUT: *Commercial Food Sanitation, Kenosha, WI, USA*
HILARY THESMAR: *Food Marketing Institute, Arlington, VA, USA*
TIMOTHY FREIER: *Merieux NutriSciences, Maple Grove, MN, USA*
KATHLEEN GLASS: *University of Wisconsin-Madison, Madison, WI, USA*

The purpose of this session is to share key learnings from food sectors that have been associated with *Listeria* outbreaks and have been conducting environmental monitoring programs (EMP) for *Listeria* spp. for more than two decades. Other sectors, such as frozen fruits and vegetables, are just starting. Even for the sectors that have been conducting EMP many years, there has been little crossover of experiences and practices between the sectors. Sectors new to *Listeria* and EMP can learn a great deal from those with experience.

S47 Stories from the Trenches: FDA Inspection after Food Safety Modernization Act (FSMA) Implementation

LILLIAN HSU: *U.S. Food and Drug Administration-CFSAN, Laurel, MD, USA*
BENJAMIN WARREN: *Land O' Lakes, Arden Hills, MN, USA*
SAMANTHA COOPER: *GMA, Washington, DC, USA*
TRAVIS CHAPIN: *University of Florida, Lake Alfred, FL, USA*
KATHY GOMBAS: *Retired CFSAN, Laurel, MD, USA*
SHARMI DAS: *U.S. Food and Drug Administration, Washington, DC, USA*

There is no doubt that the Food Safety Modernization Act is the most sweeping piece of food legislation for American manufacturers in over 70 years. The FDA has also paired updated regulations with an overhaul to their inspection program. As a result, the entire industry is on pins and needles to see how these changes will directly affect them, when their inspector comes to visit. Though the Preventive Controls for Human Food Rule's compliance deadline for large establishments, Sept. 19, 2016, will have long past by the time IAFP members storm the sunny city of Tampa for the Annual Meeting, the FDA will have had time to inspect a very small percentage of U.S. facilities. Most IAFP members will not have had exposure to the new FSMA inspection process.

FDA has outlined an inspection system that targets riskier facilities, utilizes experts for inspector and plant support, and promises a quicker turn-around for final inspection reports. These will be welcome changes and most of the industry is very eager to learn how well the new system operates. Is the sky really falling or will these changes usher-in an unprecedented period of transparency, cooperation, and streamline the FDA inspection process? We are very interested to better understand this process from first-hand accounts of those involved in the first round of FSMA inspections.

This session will directly address:

1. An FDA inspector's perspective on how the first round of FSMA inspections have gone. What are the big gaps that they've seen, and how can the industry be better prepared?
2. Challenges from the industry's perspective, a first-hand account of a site representative that has facilitated a FSMA inspection.
3. A broader view of industry feed-back from these first few months of FSMA implementation from Grocery Manufacturers Association's (GMA) membership and the GMA Science and Education Foundation.

S48 Foodborne Viruses: Detection, Risk Assessment, and Control Options in Food Processing

ALBERT BOSCH: *University of Barcelona, Barcelona, Spain, Spain*
TREVOR PHISTER: *PepsiCo, Leicester, United Kingdom, United Kingdom*
ALVIN LEE: *Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, IL, USA*

Foodborne viruses are recognized among the top rated food safety priorities in a very recent report of risk assessment experts on the identification of food safety priorities using the Delphi technique (Rowe and Bolger, 2016) and have become, over the past few years, a greater concern to the food industry. All parties agree that control measures for viruses throughout the food chain are required; however, much still needs to be understood with regard to the effectiveness of these controls and how to properly validate their performance; whether it is the personal hygiene of food handlers, the effects of processing on foods at risk, or the interpret and action on a positive test result in a virus testing program (EFSA, 2011 and FSA, 2015).

In this session, we will present the current work of an ILSI Europe expert group that provides a description of foodborne viruses, their characteristics, and responses to stress. There will, also, be a critical discussion on the technologies developed for their detection and control and the way forward on the applications for science and industry. The recommendations in this review will allow industry to perform effective control options for viruses in food processing. We will present the current state of the science in epidemiology, public health burden, risk assessment, and management options for viruses in food processing environments and will draw practical conclusions.

S49 Hepatitis E Virus: An Emerging Foodborne Pathogen?

DANIELLE YUGO: *Virginia Polytechnic Institute and State University, Blacksburg, VA, USA*
NICOLE PAVIO: *ANSES, Maisons-Alfort, France, France*
BARBARA WILHELM: *Big Sky Health Analytics, Vermilion, AB, Canada, Canada*

Recent scientific findings revealed that a significant portion of Europe's population is now seropositive for zoonotic Hepatitis E virus (HEV). Previous studies have also indicated that as much as 10% of swine liver sold in the U.S. contains zoonotic HEV. This symposium will focus on the emergence of this pathogen and its public health significance, so that we can gain a better knowledge of the diversity and epidemiology of the virus, as it relates to food safety issues in industrialized countries.

S50 Teaching for Tomorrow: Impact of School and College Food Safety Curricula on Better Informed Consumers, Career Opportunities, and the Industry Workforce of the Future

FRANCOISE FONTANNAZ: *World Health Organisation (WHO), Geneva, Switzerland, Switzerland*
JENNIFER RICHARDS: *University of Tennessee Institute of Agriculture, Knoxville, TN, USA*
CHRIS REEDY: *BioNetwork, Raleigh, NC, USA*

The need for effective food safety education in the classroom has been an ongoing discussion at IAFP for several years. A pro-active approach to more effective recruitment and succession planning has been an issue raised by the food industry at the GMA Science Forum, Leadership Forum, and other public fora, including IAFP. Although anecdotal reports have highlighted efforts, often by individuals, to raise awareness of the agri-food sector, focused discussions involving education program providers and industry stakeholders, related to food safety education in schools, have been absent from recent IAFP agendas. Experience from highly developed classroom educational programs suggests that these are leading to renewed interest in food science at the secondary and post-secondary level and to increased awareness in STEM careers. The seeds for better informed consumers, modifying food safety behaviours, and catalyzing personal exploration of STEM-based career options are planted at a young age. School-based education programs and applied community college curricula trigger interest in food science and food industry career opportunities. With engagement of appropriate stakeholders, this leads to a talented work force for the food industry, more effective succession planning, and, ultimately, safer food.

This session will directly address:

1. Proactive approaches to food science education at the school level by engaging provocative thought leaders from around the world;
2. Challenges and opportunities in food science education; raising awareness of STEM-based food industry career opportunities;)
3. Creation of training resources to educate young people as future consumers and the future workforce in safe food handling;
4. Workforce opportunities and challenges for industry; and
5. How results from this interactive session will precipitate a call-to-action! The session will engage provocative discussion with international thought leaders as speakers; utilize digital social media in the room; and solicit audience ideas and recommendations for a realistic action plan to carry this theme forward.

S51 Establishing Effective Metrics to Advance Your Food Safety Training and Education Programs

IRENE BOLAND: *Learning Development Institute, Orlando, FL, USA*
KRISTIN KASTRUP: *Alchemy Systems, Austin, TX, USA*
DAN DENNISON: *Denison Consulting and IMD, Braunau, Switzerland, Switzerland*

The food industry has made great strides in improving training and learning systems across its various sectors, but much training is still done without any structured evaluation. The need for training metrics has come up at recent IAFP meetings, but evidence-based sessions have been absent from IAFP meeting agendas. Anecdotal evidence suggests that some companies do not use any metrics to assess training effectiveness, whilst others postpone any training for new employees until after a "natural drop off period". Some of these issues are echoed in the annual Campden BRI "Global

Food Safety Training Survey" (*www.campdenbri.com*), where records of attendance are still listed amongst the most common ways of assessing training understanding and less than half of companies report any measurement of performance/behaviour after training. Clearly, a lack of effective training and education can lead to food safety management failures and this presents a risk to consumer health and brand protection. However, applying training and education programs without appropriate evaluation metrics can lead to a false sense of security.

A new focus is required for the industry to continue its journey to improve training and learning. Building on models of training effectiveness, such as the Kirkpatrick Reaction-Learning-Behavior-Results model (*www.kirkpatrickpartners.com*), this symposium considers how practices from education research can transfer to the food industry. Speakers will address how to apply training effectiveness models and share experiences about how to get the most from your training dollars, going beyond "death by PowerPoint" and counting training events.

This session will, directly, address:

1. Proactive approaches to food safety training and education evaluation;
2. How to embed suitable metrics into design of training programs; and
3. Challenges and opportunities for improving training and learning systems for effective food safety management. The session will engage audience participation, by utilizing digital social media in the room, to solicit audience reactions to ideas and recommendations.

S52 Total Diet Studies: Designs for Monitoring the Food Supply

GERALD MOY: *Food Safety Consultants International, Geneva, Switzerland, Switzerland*
MARK WIRTZ: *U.S. Food and Drug Administration, College Park, MD, USA*
KATHERINE WOODWARD: *RTI International, Research Triangle Park, NC, USA*

Chemical contaminants are an important domestic and international food safety concern, from both the health and economic perspective. A number of countries conduct a Total Diet Study (TDS) to monitor chemical contaminants (e.g. pesticide residues, industrial contaminants, radionuclides, and heavy metals), as well as nutrient levels in foods. Combined with consumption data, a TDS can provide valuable insights into dietary exposure to these contaminants and nutrients, across the total population, and in relevant sub-populations, such as children and pregnant women. A TDS provides both a snapshot, in time, of the levels of contaminants and nutrients in foods; and, if conducted regularly, information about how they change over time. Hence, a TDS can be used as:

1. a screening tool, to monitor background levels of contaminants in foods;
2. a risk management tool to help set priorities and drive actions based on public health risk; and
3. an evaluation tool to identify trends or changes over time.

TDS design may vary, depending on what information is needed, how a country's food system operates, and the resources available for data collection. This session aims to provide an introduction to the TDS as a long-term contaminant monitoring and public health tool and will describe how a TDS is conducted, as well as some of the major considerations and trade-offs that should be considered when designing a TDS. Audience members will gain a deeper understanding of how a TDS can be used to monitor chemical contaminants and nutrients in the food supply in relation to long-term, population-level exposures and how sampling design considerations, ultimately, affect the representativeness and precision of the study results.

S53 Ranking Risks in Low-resource Settings

JULIANA RUZANTE: *RTI International, Research Triangle, NC, USA*
JOHN BASSETT: *John Bassett Consulting Ltd, Bedford, United Kingdom, United Kingdom*
SARAH CAHILL: *Food and Agriculture Organization of the United Nations, Rome, Italy, Italy*

Any entity, with a responsibility for food safety from national food safety authorities to food producers and processers, needs to deal with numerous food safety issues, often simultaneously, and with limited resources. Therefore, it is becoming increasingly important to rank and prioritize food safety efforts in a structured and scientific manner, so that resources can be allocated to most efficiently protect public health. In recent years, numerous approaches to rank food safety risks have been published and some have been implemented by national authorities and international organizations to better inform risk management decisions. However, developing and implementing a risk ranking approach is still a significant challenge for many countries, particularly those with limited resources. Some of the challenges relate to the multitude of methods and tools available to rank risks, the lack of data, and the lack of expertise needed to conduct those types of efforts. This symposium aims to, directly, address this issue. It will present and discuss a proposed approach for ranking risks in poor and low resource settings and will, also, look at selected examples of how ranking is being approached in countries where technical or financial resources, or both, are lacking.

S54 Tools to Improve Interactive Food Safety Training for Small Food Facilities

KAIPING DENG: *Institute for Food Safety and Health (IFSH), Lisle, IL, USA*
ANGELA SHAW: *Iowa State University, Ames, IA, USA*
OMAR OYARZABAL: *University of Vermont Extension, South Burlington, VT, USA*

New and established food safety regulation are complex and difficult to understand by mid-size to small-size farmers/processors. In addition, many mid to small-size farmers/processors are in remote locations where access to training facilities is limited. This symposium highlights the current efforts, by different constituents, on how to respond to the training needs of mid- to small-size processors, in regard to the current food safety regulations. The emphasis of this symposium is, not only, to highlight the initiatives to provide training related to the latest food safety regulations (e.g., FSMA), but to, also, review the current approach to fulfill the training associated to already establish regulations (e.g,. HACCP). This symposium includes the participations of members of the Applied Laboratory Methods and Food Safety Education Professional Development Groups.

S55 Translating the Big Data to the Food Industry

SURESH D. PILLAI: *Texas A&M University, College Station, TX, USA*
RAMIN KHAKSAR: *Clear Labs Inc., Menlo Park, CA, USA*
ANTONIOS ZOGRAFOS: *SafeTraces, Livermore, CA, USA*

The food industry needs rapid, robust pathogen detection and characterization technologies that yield useable information, in addition to presence or absence tests, only. The advent of powerful molecular tools combined with advances in computational technology has created a virtual tsunami of data. However, there are still challenges about how to analyze, interpret, and communicate the omics results, in a way that is more understandable and applicable for the food industry. The data is still too complex for the food industry to utilize, effectively. There is a disconnect between what the science

offers and what the industry can adopt. The current gaps between technology potential and industry adoption will be highlighted. This symposium explores the nuances of integrating omics into the food industry in the industry's quest to ensure safety, quality, and transparency.

S56 Chemical and Microbial Risk Assessment: Similarities and Differences

JOSEPH RODRICKS: *Ramboll Environ, ARLINGTON, VA, USA*
ROBERT BUCHANAN: *University of Maryland, Department of Nutrition and Food Science and Center for Food Safety and Security Systems, College Park, MD, USA*
JANE VAN DOREN: *U.S. Food and Drug Administration, College Park, MD, USA*

In food safety, risk assessment is the scientific evaluation of known or potential health effects resulting from human exposure to chemicals or microorganisms. Exposure to pathogenic microorganisms is different from exposure to chemicals. Microorganisms differ from chemicals, as they can grow or die and the level of exposure to microorganisms can change, dramatically, in a short period of time. The speakers in this session will discuss the similarities and differences between chemical and microbial risk assessment; risk-risk and risk-benefit analysis. Some case studies will, also, be presented.

S57 Foodborne Outbreak Updates

KARI IRVIN: *U.S. Food and Drug Administration, CORE, CFSAN, College Park, MD, USA*
BONNE KISSLER: *U.S. Department of Agriculture-FSIS, OPHS, AES, Atlanta, GA, USA*
MICKEY PARISH: *U.S. Food and Drug Administration–CFSAN, College Park, MD, USA*
ALVIN CROSBY: *U.S. Food and Drug Administration, Greenbelt, MD, USA*
EVELYN PEREIRA: *U.S. Food and Drug Administration, College Park, MD, USA*

This session will summarize recent North American and international outbreaks of hepatitis A virus (HAV) associated with imported frozen berries and challenges for agencies that have investigated and responded to the outbreaks. A second speaker will describe the investigation of a *Salmonella* I 4,[5],12:I outbreak linked to rotisserie pork and chicken and the contributing factors that were identified. The recent *Listeria* outbreak involving frozen raw vegetables has generated policy challenges for improved outbreak detection and response from foods that have not historically been implicated in outbreaks. A speaker will describe the challenges and actions that have been taken. Additionally, this session will provide a platform for a brief summary of two other recent outbreak investigations: one speaker will describe the investigation of *E. coli* O157 outbreak in soy nut butter and another speaker will describe the investigation of *Listeria monocytogenes* in soft cheese. Both speakers will touch on the collaboration between federal, state, and local health agencies and actions taken to protect public health, as well as highlight investigational findings at the manufacturing firms that may have led to contamination of product.

S58 Fresh Produce-Pathogen Pairs in the U.S. and Europe

CRAIG HEDBERG: *University of Minnesota, School of Public Health, Minneapolis, MN, USA*
CHRISTOPHER BAKER: *University of Florida, Gainesville, FL, USA*
ANA ALLENDE: *CEBAS-CSIC, Espinardo, Spain, Spain*

The environmental factors that may explain why certain pathogens are found in certain fresh produce in the United States and Europe continue to be investigated. There are many unknown factors that influence why certain pathogens are associated with fruits and vegetables in certain environments. This session will include perspectives from an expert with experience in fresh produce outbreak investigations and discussions on how agencies determine the source of pathogens during fresh produce outbreaks. The importance of source tracing during fresh produce outbreak investigations will be highlighted. Foodborne outbreak surveillance systems and data are limited in many regions throughout the global produce chain. The most recent global fresh produce-pathogen pair data (with an emphasis on the U.S. and Europe) will be presented to highlight the extent of the problem with certain pathogens and fresh produce commodities. A fresh produce and environmental microbiology expert will present current research on the main factors affecting the bacterial community of fresh produce and the potential consequences on produce safety. The role of phyllosphere-associated bacteria of fresh produce and how microbial population dynamics affect the prevalence of pathogens on fresh produce will be highlighted. Lastly, presenters will discuss future research strategies to reduce the burden of foodborne illness due to fresh produce. With the many risks associated with fresh produce, a discussion of the major factors that influence produce-pathogen pairs in different production chains, environments, and systems in the U.S. and Europe is warranted.

S59 Combatting Bioterrorism: How Select Agent Testing Laboratories are Staying One Step Ahead of the Bad Guys

RANDAL LAYTON: *Food Emergency Response Network, USDA-FSIS, Athens, GA, USA*
MICHAEL PERRY: *New York State Department of Health, Saratoga Springs, NY, USA*
STEVE WEAGENT: *Weagant Consulting, Poulsbo, WA, USA*

The ability to detect select agents and toxins is becoming increasingly imperative in today's global food industry. Diseases caused by select agents are not only a public health issue, but also a problem of national security. With a larger vulnerable population than ever before, the detection of biological agents create a gap in the food defense system where everyone requires sustenance. This session will include three speakers that will present topics on the detection of select agents in foods. An overview of current research and method development projects, large scale surveillance, proficiency testing, and high profile event analysis will be presented by the Food Emergency Response Network. Current research areas on method development for the detection of various select agents in foods will, also, be covered. Another presentation will include identifying *Clostridium botulinum* and Ricin toxin in foods by Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF/MS). This method utilizes the endoproteinase activity of the toxin to identify all BoNT types with a MALDI-TOF/MS by cleaving peptides at specific sites. Each toxin is identified by the mass-to-charge ratios of fragmented peptides for BoNT and the depurination of a RNA substrate for ricin toxin. The final speaker will discuss the development of an enrichment broth that supports the outgrowth of *Yersinia pestis*, a differential chromogenic agar to detect *Y. pestis* in a background of competitors naturally present in foods, as well as screening and identification techniques to verify isolates. This speaker will, also, elaborate on the development of a rugged and specific real-time PCR method for screening food enrichments for presence of *Y. pestis* and suspect colonies.

S60 A Paradigm Shift in Understanding and Controlling *Salmonella* of the Future

YOUNG MIN KWON: *University of Arkansas, Fayetteville, AR, USA*
STEVEN RICKE: *University of Arkansas, Fayetteville, AR, USA*
MEGAN BEHRINGER: *Indiana University, Bloomington, IN, USA*

Salmonella has been a major foodborne pathogen of concern for several years and scientists have worked relentlessly to reduce its incidence. Several initiatives such as antimicrobial interventions, process control, and pre-harvest food safety, among others, have helped to improve *Salmonella*-related food safety. However, after years of researching and understanding *Salmonella*, foodborne illnesses due to the pathogen continue to hound the food industry and scientists. The pathogen is being isolated from novel niches and has developed antibiotic resistance and tolerance to food processes. *Salmonella* is truly an ever-evolving pathogen, and the current approaches will not be adequate to control it in the future.

A paradigm shift in the thought process to understand the pathogen and in designing strategies is needed to ensure improved *Salmonella* food safety in the future. Modern methods such as Whole Genome Sequencing are helping researchers to take a deeper look at the organism, while the increased emphasis on microbiomes is providing clues about occurrence/survival of *Salmonella* in its many niches. Some scientists have developed novel, rapid, and accurate methods for pathogen detection to prevent contaminated food from entering commerce; similarly, the industry is modifying their *Salmonella* control programs to improve food safety. The scientific community should use these tools to address the existing gaps in the knowledge of *Salmonella* to achieve a higher degree of food safety.

The objective of the symposium is to provide novel perspectives and futuristic concepts on the ever-evolving *Salmonella* and on designing control strategies. The symposium aims to create discussion on missing crucial information on *Salmonella*, possible new directions for research, and addressing knowledge gaps that are preventing us from achieving food safety related to *Salmonella* and microbiome-*Salmonella* interactions, among others.

S61 Foodborne Parasites in Organic and Conventional Agricultural Practices: Food Safety Issues That Can Affect Your Mind

HELEN MURPHY: *U.S. Food and Drug Administration–CFSAN, Office of Applied Research and Safety Assessment, Laurel, MD, USA*
TEODOR POSTOLACHE: *University of Maryland, Baltimore, MD, USA*
ROBERT COWIE: *University of Hawaii, Honolulu, HI, USA*

Organic agriculture is a growing industry that can be a profitable, sustainable business for producers, globally. The rapid growth of this sector has triggered different discussions and concerns about the impact of organic agriculture on the safety of foods in comparison to conventional agriculture. In addition to bacterial and viral pathogens, foodborne parasites, especially those that cause intestinal and neurologic disease, represent a concern considering the epidemiologic data that shows elevated risk for acquiring parasitic infections through consumption of different food commodities. Cyclospora cayetanensis has been associated with large outbreaks in the U.S. and Canada. Severe cases of eosinophilic meningitis caused by the foodborne parasite Angiostrongylus cantonensis have been epidemiologically linked to exposure to leafy greens or edible mollusks, worldwide. Toxoplasma gondii, another important foodborne parasite, is associated with behavioral dysregulation and neuropsychiatric conditions that may go undiagnosed for years and result in increased morbidity and mortality of consumers at risk. This symposium will explore the current and past data, regarding parasitic diseases, that can be linked to consumption of food commodities produced by organic and conventional agricultural practices with impact on consumer's health. Speakers from federal U.S. agencies and from academia will discuss these topics.

S62 Water for Food Processing Falls in the Crack Between RTCR (Revised Total Coliform Rule) and FSMA

JOEL STOKDYK: *U.S. Geological Survey, Wisconsin Water Science Center, Laboratory for Infectious Disease and the Environment, Marshfield, WI, USA*
VINCENT HILL: *Centers for Disease Control and Prevention, Division of Foodborne, Waterborne and Environmental Diseases, Atlanta, GA, USA*
WILLIAM SHAW: *U.S. Department of Agriculture-FSIS-OPPD, Washington, DC, USA*
JULIE JAVIER: *U.S. Environmental Protection Agency, Arlington, VA, USA*
PHYLLIS POSY: *Strategic Services & Regulatory Affairs Atlantium Technologies, Har Tuv Industrial Park, Israel, Israel*

This session will confront the generally accepted position that if water is good enough to drink, it is good enough to use for food processing. Speakers will analyze the data on whether/how the gap between EPA Drinking Water and FDA FSMA policies leave a hole in the middle that can compromise food safety and dialogue about solution models. The Revised Total Coliform Rule (RTCR; effective 4/2016) refocuses the Safe Drinking Water Act criteria on fecal *Escherichia coli* as the indicator for fecal contamination, and total coliforms as the indicators of pathways through which contamination can enter the system. Public systems will be required to do an assessment if their sampling results indicate that they are vulnerable to contamination. It changes requirements for public notification when samples are positive in favor of triggered assessments ("find and fix"). Users may not be informed that microbially contaminated water was provided, especially if the system implemented a fix or even a plan for a fix within the required time frame. While impact on individuals drinking might be minimal, food processors who rely on municipal water could be contaminating their products. Municipal water can be used for processing (food contact or even ingredient water) without any risk assessment because FSMA specifically excluded municipal water users from requirements to address water in their written Food Safety Plans. In reality, some systems served exclusively from ground water supplies are not disinfected. Recent research in Minnesota (where 567 groundwater systems were compliant and did not disinfect) found EPA compliant water, when not disinfected, can be contaminated with *Salmonella* and other organisms of concern. Viable pathogens can intrude, through non-point sources or through the pipe and pump hydraulics, and go undetected by monitoring under the Revised Total Coliform Rule. Our current statistics do not capture the link between food outbreaks where the underlying transmission agent or amplification is in the "drinking water". Here, government panelists discuss: How big is the hole in the middle and can it compromise food safety? What should we do about it?

S63 Staying Ahead of the Curve: Food Allergen Contamination and Recalls in Today's Global Food System

STEVEN GENDEL: *IEH Laboratories and Consulting Group, Rockville, MD, USA*
PETER BEN EMBAREK: *World Health Organization/INFOSAN Network, Geneva, Switzerland, Switzerland*
DONALD JONES: *Atkins Nutritionals, Inc, Denver, CO, USA*

In the past several years, both the U.S. and Europe have experienced increases in the number of food recalls. According to the Food Allergy Research and Resource Program (FARRP), food allergen recalls of USDA and FDA regulated products are on the rise. Two recent incidences, in 2015 and 2016, represent the nature and magnitude of possible allergen contamination of commodity products and their impact across borders. They, also, bring home the urgency to better understand the practices at primary production that may lead to allergen contamination of ingredients and the need to get ahead of the next allergen recall, locally and internationally. Lastly, they illustrate the challenges faced by food safety professionals to provide a safe product and the impact to consumer perceptions about food safety. This session will examine current supply chain operations by leveraging lessons learned from past experiences. Speakers will provide tangible recommendations and best practices that address allergen management, recalls, and public communications; procurement; and global growing conditions. At the end of this session, attendees will be able to incorporate learnings into existing food safety and food recall readiness plans to enhance safety and to protect the health of today's consumer.

S64 A Roadmap to Food Allergy Safety: A Consensus Report from the National Academies of Sciences, Engineering, and Medicine

VIRGINIA STALLINGS: *Children's Hospital of Philadelphia, Philadelphia, PA, USA*
SHARON DONOVAN: *University of Illinois, Urbana, IL, USA*
STEVE L. TAYLOR: *Food Allergy Research & Resource Program, Department of Food Science & Technology, University of Nebraska, Lincoln, NE, USA*

Nearly 15 million people are affected by food allergies in the United States, alone; and current global trends show that this number of individuals is increasing, particularly in developed countries. A committee of the National Academy of Sciences, Engineering and Medicine was charged with examining critical issues related to food allergy, including:

1. the prevalence and severity of food allergies in the United States and globally and its impact on affected individuals, families, and communities;
2. current understanding of food allergies as a disease; and
3. in diagnostics, treatments, prevention, and public policy.

This consensus study engaged a broad array of stakeholders, including government agencies, organizations, academic institutions, industries, policy makers, and patient organization groups, in addition to bringing together leading investigators from relevant fields, clinicians, and parents to engage in review of the issues surrounding food allergies.

This symposium, first, highlights briefly, the committee's recommendations (not regulations) for future directions in several key areas: steps to increase public awareness of food allergy; promoting research on both disease causation and management; informing preventive approaches to food allergies; and identifying research gaps and making recommendations to fill them. Secondly, the symposium will expand on the findings and recommendations of the committee in the area of food allergy prevention. Finally, the symposium will describe what are the food scenarios and settings with the highest risks and management approaches. The last presentation will convey findings and recommendations related to the status of assessing allergen thresholds in individuals, reference doses, and their use in labeling and management of food allergies.

S65 What is the Meaning of Zero Tolerance in the Age of Food Genomics?

MATTHEW RANIERI: *Acme Smoked Fish Corporation, Brooklyn, NY, USA*
DOUGLAS MARSHALL: *Eurofins Scientific Inc., Fort Collins, CO, USA*
PALMER ORLANDI: *U.S. Food and Drug Administration, Silver Spring, MD, USA*

Finding specific gene sequences in a DNA extract from foodstuffs or cultural enrichments can have many meanings. Modern DNA sequencing and bioinformatics allow the determination of specific genes (e.g., targeted bacterial microbiomes) or total DNA sequences (metagenomes) in matrices including food, which has not been culturally enriched. This new paradigm of food analysis brings us to question whether finding a specific gene sequence should be considered, [a] a true-positive or viable pathogen; [b] an indicator of the potential for viable organisms; [c] a history of past pathogen contamination; or [d] DNA from non-viable cells. Zero tolerance rules for *Listeria monocytogenes* and *Escherichia coli* O157 are predicated on evidence from finding a specific DNA amplicon and/or culturing and isolation of the target organism. In early days of PCR tests, based on presence/absence of a specific PCR amplicon and not a defined sequence, the dilemma of PCR positive/culture negative samples was addressed and seemingly rectified. But, since some pathogens appear to be ubiquitous within food production environments, what does finding their DNA signature in a microbiome or metagenome mean? Does merely finding a sequence constitute a basis for action? Will we reach a point in which zero tolerance rules lose meaning as we are able to analyze effectively larger sample sizes just from their DNA extract; thereby, increasing chances of finding pathogens in a production environment? Should we begin transitioning from zero tolerance pathogen rules to quantitative standards? These are key questions, in the early days, of applying genomics to microbial food analysis. Here we discuss the implications of using microbiomes beyond straight presence/absence PCR. The emphasis of this symposium will be on how genomic technologies could potentially call into question the meaning of zero tolerance and finding pathogen sequences. A successful outcome will provide guidance for practitioners of microbiomics for industry and regulatory problems.

S66 Challenges and Strategies in Detecting Foodborne Pathogens in Low-water Activity Foods

JOSHUA GURTLER: *U.S. Department of Agriculture-ARS, Eastern Regional Research Center, Wyndmoor, PA, USA*
SHABARINATH SRIKUMAR: *University College Dublin, Dublin, Ireland, Ireland*
JUNIA JEAN-GILLES BEAUBRUN: *U.S. Food and Drug Administration, Laurel, MD, USA*
MONICA PONDER: *Virginia Tech, Blacksburg, VA, USA*
FATMEH KOBAISSI: *MEFOSA-MENA, Hamnra Beirut, Lebanon, Lebanon*
JULIE ANN KASE: *U.S. Food and Drug Administration, College Park, MD, USA*

Low-water activity (aw) and dried foods, such as grain-based and dried ready-to-eat cereal products, powdered infant formula, peanut and other nut pastes, flours, and spices have been increasingly associated with product recalls and foodborne outbreaks due to contamination by pathogens, such as *Salmonella* spp. and enterohemorrhagic *Escherichia coli*. In particular, recent foodborne outbreaks and product recalls related to *Salmonella*-contaminated spices and *E. coli* in flour have raised the level of public health concern for spices or other dry foods as agents of foodborne illness. Foodborne pathogens, in low aw foods, often exhibit an increased tolerance to heat and other intervention treatments that are lethal to cells in high aw environments. It is increasingly challenging to eliminate these pathogens in many dry foods or ingredients without impairing organoleptic quality. Some current control or preventive measures are based on hygienic design, zoning, and implementing efficient cleaning and sanitation procedures. Improved, rapid bacteriological resuscitation and detection assays for foodborne pathogens that are in very low numbers and should be developed. Foodborn pathogen detection challenges in dry products (e.g., spices, nuts and flour) and their processing environments will be discussed and some of the current methodologies that may help overcome these challenges will be identified.

S67 Preventive Controls Other Than CCP: Choosing, Verifying, and Validating

LORALYN LEDENBACH: *Kraft Heinz Company, Glenview, IL, USA*
BALASUBRAHMANYAM KOTTAPALLI: *Conagra Brands, Omaha, NE, USA*
RICHARD BROUILLETTE: *Commercial Food Sanitation, South Burlington, VT, USA*
MAILE HERMEIDA: *Hogan Lovells US LLP, Washington, DC, USA*

CFR 117.135(a)(2) of the Preventive Controls for Human Food rule states that preventive controls include: "(i) Controls at Critical Control Points (CCPs), if there are any CCPs"; and "(ii) Controls, other than those at CCPs, that are also appropriate for food safety." There is considerable interest and some confusion among food safety professionals as we debate and attempt to decide the appropriate preventive controls for a manufacturing facility. Decisions on how to choose, verify, and validate "controls other than CCPs" can be difficult, especially for smaller companies. This symposium will serve as an interactive session for industry to share practices and lessons learned for decisions on CCPs, Operational Prerequisite Programs (OPrPs), and Prerequisite Programs (PrPs); how to identify them, how to implement them, and how to verify and validate them where applicable. Speakers will discuss the various decision trees that can be used to determine different types of preventive controls based on hazard analysis, practical examples and considerations for their choices, verification and validation options for these types of controls, and a discussion on regulatory implications of preventive control choices. Attendees should feel free to start discussions about these examples, to collectively benchmark current practices among participants.

S68 The National Antimicrobial-resistance Monitoring System: Twenty Years of Vigilance

PATRICK MCDERMOTT: *U.S. Food and Drug Administration, Laurel, MD, USA*
CINDY FRIEDMAN: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
SHAOHUA ZHAO: *U.S. Food and Drug Administration, Laurel, MD, USA*
UDAY DESSAI: *U.S. Department of Agriculture-FSIS, Washington, DC, USA*

Antibiotic resistance is one of the foremost global health challenges. Surveillance is an essential component of a coordinated national strategy to combat antimicrobial resistant bacteria. The year 2016 marks the 20th anniversary of the National Antimicrobial Resistance Monitoring System (NARMS). This interagency public health surveillance system is a collaborative effort of the USDA, CDC, and FDA that tracks antibiotic resistance in foodborne bacteria. In its twenty years of work, NARMS has provided foundational data for understanding the burden of resistant enteric infections and has described trends in resistance that has been pivotal for regulatory decision making. In the process, detailed microbial data for over 160,000 bacterial strains has been placed in the public domain, for use by others. In addition, NARMS scientist have made substantial contributions to advance the field of resistance surveillance, developing the first methods for monitoring antibiotic resistance in *Campylobacter* spp., publishing the largest studies on the use of whole genome sequence data for predicting resistance, and developing molecular assays to help associate resistant bacteria with their source, while also supporting international efforts to build surveillance capacity around the world. With new rules governing the use of antibiotics in food animals beginning in 2017, NARMS will continue its work by monitoring the impact on resistance of this and other interventions. New advanced DNA sequencing technologies are greatly enhancing the data being generated in NARMS. The goal of this symposium is to present the past, present, and future of NARMS, with the aim to illustrate its role in protecting public health and the potential advances made possible by new approaches to surveillance.

S69 Empowering Food Laws in Emerging Economies

GERALD MOY: *Food Safety Consultants International, Geneva, Switzerland, Switzerland*
ATEF IDRISS: *MEFOSA, Beirut, Lebanon, Lebanon*
MARIA LOVELACE-JOHNSON: *Food and Drug Authority, Accra, Ghana, Ghana*
VINCENT HEGARTY: *Michigan State University, Dearborn, MI, USA*

As the administration, economies, and infrastructure of developing nations change, it is important for regulations to evolve, accordingly. Developing economies tackle issues of sustainability and food security, as well as food safety. Here it is important safety does not take a back seat. Empowering regional CODEX committees, professional introduction of new laws, and continuous harmonization with regional economies can limit inter-ministerial feuds at the national level, impediments in regional trade, and poor harmonization with global preventive food safety initiatives. This symposium will address consumer needs and impediments that have been affecting food safety laws in emerging economies of Ghana, Lebanon, and Vietnam, as well as the steps that have already been taken to overcome some of these obstacles, including supporting regional CODEX committees, training on risk based standardization, addressing regulatory frameworks and poor enforcement practices.

S70 Microbiological Safety of Unpasteurized Fruit and Vegetable Juices Sold in Juice Bars and Small Retail Outlets

ARMITRA JACKSON-DAVIS: *Alabama A&M University, Madison, AL, USA*
AUBREY MENDONCA: *Iowa State University, Ames, IA, USA*
DAVID WHITMAN: *CFSAN Office of Food Safety, San Diego, CA, USA*
LAWRENCE GOODRIDGE: *McGill University, Montreal, QC, Canada, Canada*

Non-traditional juices are those fruit and/or vegetable juices that are minimally processed and might not be subjected to heat pasteurization, like traditional commercial juices. Unpasteurized juices fall within this category and have a shorter shelf-life when compared to traditional juice products. Unpasteurized juices are sold in juice bars or retail stores and are popular with consumers due to the belief that these products are healthier compared to traditional commercial juices. Additionally, they are typically less acidic than traditional juice products and do not fall under Juice HACCP regulations requiring that producers demonstrate a five-log reduction of pertinent pathogenic bacteria. Consequently, these products lack the built-in protections that are provided for traditional juices. Considering the rapid growth in the number of juice bars and very small producers serving raw juices, the microbial safety of unpasteurized juices warrant serious attention. Recently documented outbreaks, involving unpasteurized juice contaminated with Hepatitis A virus, further substantiate the need for discussion of safety issues. This symposium will bring together personnel from academia (United States and Canada) and the U.S. Food and Drug Administration to provide perspectives on the microbial safety of juices consumed raw, including regulatory implications, challenges in ensuring juice safety, and potential solutions.

S71 Advancing Food Safety Internationally through the Use of Innovative Technologies: Food Irradiation

ROBERT TAUXE: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
MARTIN DUPLESSIS: *Bureau of Microbial Hazards, Food Directorate, Health Canada, Ottawa, ON, Canada, Canada*
RONALD EUSTICE: *Food Irradiation Newsletter, Tucson, AZ, USA*
YAOHUA (BETTY)
FENG: *University of California-Davis, Davis, CA, USA*

Food irradiation is a promising food safety technology that can eliminate disease-causing microorganisms in food. The 2016 announcement of Canada's intention to approve irradiation of ground beef plus the growing use of irradiation as a phytosanitary treatment for fruits suggests that the barriers to use of this technology might be lowering. This symposium will provide an international perspective on food irradiation by summarizing the recent policy, research, and marketplace activities.

The first speaker, Robert Tauxe from the U.S. Centers for Disease Control and Prevention, will provide an overview of the use of novel technologies, including irradiation, to reduce the frequency of foodborne illness. Next, Martin Duplessis from Health Canada will describe the potential advance in food safety that irradiation of beef could provide. He will provide an overview of the data that lead to the proposal to permit irradiated ground beef in the marketplace. Ronald Eustice, Food Irradiation Newsletter, will provide an overview of the growing national and international marketplace presence of irradiated meat and produce. Yaohua (Betty) Feng, University of California, Davis, will present messages that work in the marketplace based upon her research and Wegmans supermarket experience as relayed by Kathleen O'Donnell.

The session is designed to facilitate a dialogue informed by an overview of the current international policy and consumer research on food irradiation and will focus on advancing food safety innovative technologies to the next level, while ensuring that appropriate educational messages are developed for consumers and industry stakeholders.

S72 Social Responsibility's Influence over Food Safety and Quality

RUTH WOIWODE: *Food Safety Net Services, San Antonio, TX, USA*
LARRY LICHTER: *McCormick, Baltimore, MD, USA*
BOBBY KRISHNA: *Dubai Municipality, Dubai, United Arab Emirates, United Arab Emirates*
JEFFREY KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*

The concept of Social Responsibility has saturated social media over the last few years and, finally, crept into our corporate/organizational pillars. In its infancy, green/responsibility initiatives were often placed with the quality leaders; though today, many large companies have their own CSR (Corporate Social Responsibility) Departments. CSR encompasses a variety of facets: environmental conservation, community outreach/volunteerism, sustainable sourcing/animal welfare, good labor practices, etc.

Does social responsibility have any effect on food safety and quality, beyond a need to "do the right thing"? Together, we will examine several examples where these responsibility facets have a direct connection to vital food safety and quality concerns in the food industry, today.

This session will, directly, address:

1. animal Welfare initiatives, which have a significant effect on quality of the final products;
2. responsible sourcing in developing countries, which is vital to food safety of exotic raw materials;
3. whether shelf-life dates really necessary or the biggest contributor to food waste;
4. whether we are using too much water during sanitation; and
5. whether water conservation initiatives lead to a cleaner facility.

Each speaker will briefly introduce their Social Responsibility topic and, then, explore the relationship between it and food safety or quality outcomes.

S73 Toward Risk-based Microbial Standards for Irrigation Water

KELLY BRIGHT: *University of Arizona, Tucson, AZ, USA*
ELISABETTA LAMBERTINI: *RTI International, Rockville, MD, USA*
WILL DANIELS: *Will Daniels Consulting Group, Carmel Valley, CA, USA*
KRUTI RAVALIYA: *U.S. Food and Drug Administration, College Park, MD, USA*

The microbial quality of irrigation water used in edible crop production has been the object of increasing scrutiny. However, the actual risk of pathogen contamination posed by irrigation water, as well as the relative risk compared to other factors, are not well understood or quantified.

This symposium aims to critically assess available evidence on the role of microbial irrigation water quality in (a) introducing pathogens into produce fields and (b) directly contaminating produce units. The symposium will address the wider context of other contamination and cross-contamination processes that may occur in the field and are affected by water (e.g., splashes of animal feces), but will focus on the fate and relative importance of pathogens occurring in source waters or distribution systems.

Goals of the symposium include presenting or addressing:

1. a summary of available data on microbial quality, in particular bacterial and viral, of irrigation waters in the U.S. (at sources, at the point of delivery, and evidence of direct water-produce transfer), including data gaps that hinder the development of effective management standards;
2. the distinction between direct and indirect role of irrigation water in introducing and spreading pathogens onto the field, and the relationship between irrigation and other processes affecting pathogen ecology (e.g., conditions for bacterial growth or survival). The latter will not be the focus of this symposium, but needs mentioning;
3. a quantitative risk-based framework for assessing the impact of irrigation water quality on the likelihood and extent of produce contamination; and
4. feasibility and constraints of potential risk-based regulatory and management approaches.

S74 Root Cause Analysis

KARIN HOELZER: *The Pew Charitable Trusts, Washington, DC, USA*
LAURA BROWN: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
TIMOTHY JACKSON: *Nestle USA, North America, Glendale, CA, USA*
JENNY SCOTT: *U.S. Food and Drug Administration–CFSAN, College Park, MD, USA*

Root cause analysis: what is it and how is it being used in the food safety world? Root cause analysis is an application of the broader field of systems analysis. Systems analysis is the process of understanding all aspects of a problem, in order to fully address the problem. The root causes are the underlying reasons for a problem. HACCP is another application of systems analysis as a problem prevention approach. The term root cause analysis is being used more often in the context of food safety, but it likely has many different meanings depending on who and where it is spoken of. This symposium will explore the use of root cause analysis in the food safety world and discuss the need to harmonize the meaning, to mitigate confusion about the term and improve application of the approach. Three speakers representing outbreak investigations, industry, and regulatory programs will describe what the term means to them and how they apply it.

SF1 Predictive Microbiology and Risk Assessment Tools

FANNY TENENHAUS-AZIZA: *CNIEL (French Dairy Board), PARIS, France, France*
MARIEM ELLOUZE: *Nestlé, Lausanne, Switzerland, Switzerland*
YUHUAN CHEN: *U.S. Food and Drug Administration, College Park, MD, USA*
PANAGIOTIS SKANDAMIS: *Agricultural University of Athens, Athens, Greece, Greece*
FERNANDO PÉREZ-RODRÍGUEZ: *University of Cordoba, Cordoba, Spain, Spain*
YVAN LE MARC: *ADRIA Développement, Quimper, France, France*
MARK TAMPLIN: *Food Safety Centre, Tasmanian Institute of Agriculture, University of Tasmania, Hobart, Australia, Australia*

The International Committee on Predictive Modelling in Foods has organized a symposium for the IAFP 2017 Annual Meeting to encourage the use of predictive microbiology and risk assessment tools by food business operators. These tools can help solve industrial incidents, prove compliance with microbiological criteria, or justify the use of specific control measures in the food chain. Such tools are, more and more, used by authorities to assess the exposure to microbiological contaminants and to support management decisions. This symposium will help microbiologists and stakeholders from the food sector gain knowledge and expertise on how to use these predictive modeling and risk assessment tools, based on oral presentations. Software, to be presented, will offer different features: databases, growth/no growth interfaces, growth and inactivation fitting tools and predictors, and risk assessment tools. Following an introductory talk, five tools will be presented, orally. Their developers will describe the different features, the possible applications, overviews of the interfaces, and share brief case studies.

As a complement to the oral presentations, there will be a live demonstration or "software fair", during which attendees can come in and try out the various tools, in a friendly, hands-on session with the developers. Details for the "software fair" can be found under Special Events (#3977).

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Roundtable Abstracts

RT1 Starter Cultures as a Natural Antimicrobial to Improve the Safety of Ready-to-Eat Food

DAVID B. SCHMIDT: *U.S. Department of Agriculture, Leesburg, VA, USA*
ABIGAIL SNYDER: *The Ohio State University, Columbus, OH, USA*
ALEX BRANDT: *Food Safety Net Services, San Antonio, TX, USA*
VERONIQUE ZULIANI: *CHR HANSEN, Arpajon, France, France*
PETER TAORMINA: *Club Chef LLC, Cincinnati, OH, USA*
KATHLEEN GLASS: *University of Wisconsin-Madison, Madison, WI, USA*

Microorganisms are traditionally used to carry out fermentation processes. For thousands of years, mankind has used them in the processing of fish, meat, and vegetables, as well as to make food products such as bread, beer, wine, vinegar, yoghurt, and cheese. Fermentation is, thus, one of the oldest food processing transformation and conservation techniques. This biological process not only improves the nutritional and organoleptic qualities of the food (taste, appearance, smell, texture), but adding microorganisms will, also, favor desirable flora, to the detriment of undesirable flora, thereby preventing spoilage, inhibiting pathogens, and increasing safety. The goal of this roundtable is to gather all major stakeholders involved in food safety, including manufacturers, food authorities, scientists, ingredient suppliers, retailers, etc. and to discuss the concept of bioprotection through the use of starter cultures in ready-to-eat (RTE) food (smoked salmon, meat, salad, etc.). Advantages (inhibition of pathogenic bacteria, clean labelling, etc.), hurdles (regulation, labelling), and new scientific insights will be discussed. Recent studies have demonstrated the strong efficiency of lactic acid bacteria to inhibit the growth of *Listeria monocytogenes,* while not modifying the sensory attributes of senstive foods as diverse as cooked ham, lettuce, and smoked salmon; i.e., food that are not traditionally considered as fermented food. The goal of this session is to share the lastest scientific results dealing with bioprotection of food and to present examples of RTE food, already available on the market, that contain bioprotective cultures. Safety of protective cultures, labelling rules, and consumer acceptance will, also, be discussed.

RT2 Hear All About It: Managing a Crisis

THEODORA MORILLE-HINDS: *Kellogg Company, Battle Creek, MI, USA*
RYAN NEWKIRK: *U.S. Food and Drug Administration, College Park, MD, USA*
JENNIFER PIERQUET: *Iowa Dept of Inspections & Appeals, Des Moines, IA, USA*
ANN MARIE MCNAMARA: *Target, Minneapolis, MN, USA*
MICHAEL ROBERSON: *Publix Super Markets, Inc., Lakeland, FL, USA*
HAL KING: *Public Health Innovations LLC, Fayetteville, GA, USA*

In recent years corporate crises have overwhelmed the headlines. Food manufacturers, grocery retailers, and restaurants have been impacted by supply chain disruptions, natural and man-made disasters, such as terrorism, operational malfunctions, and economically-motivated adulteration, leading to numerous food recalls and foodborne illnesses, as well as companies shutting down operations, either temporarily or permanently.

Understanding what can be done to prepare for, respond to, limit the impact of, and recover from a crisis is a necessity to protect consumer health and a company's brand and reputation. The panel will discuss:

- Techniques and tools to help prepare for and respond to a crisis
- Critical elements of a crisis management plan, including communication strategies
- Regulations affecting the industry and how companies are pivoting their strategies to react
- The importance of working with local and federal agencies during a crisis
- How companies can recover from a crisis and remain open to serve the public in the midst of a crisis

RT3 Seafood-associated Vibriosis: Turning the Trend Around

KEN MOORE: *Interstate Shellfish Sanitation Conference, Columbia, SC, USA*
SALINA PARVEEN: *University of Maryland Eastern Shore, Princess Anne, MD, USA*
CHRIS SCHILLACI: *Massachusetts Division of Marine Fisheries, New Bedford, MA, USA*
JOHN JACOBS: *NOAA, Oxford, MD, USA*
WILLIAM WALTON: *Auburn University, Dauphin Island, AL, USA*
ERIN BURDETTE: *Centers for Disease Control and Prevention, Atlanta, GA, USA*

Vibrio spp. are a predominant cause of seafood-associated illnesses, globally, and vibriosis in one of the few foodborne diseases that has trended upwards in the United States. As Vibrio spp. are naturally ubiquitous organisms in the marine environment, strategies for mitigation of this hazard are particularly challenging. Vibriosis is primarily associated with the consumption of molluscan shellfish, which is regulated through the National Shellfish Sanitation Program (NSSP). Changes in the NSSP approach to mitigating the risk of vibriosis will be discussed, including the emerging challenge of shellfish aquaculture. However, there are other sources of Vibrio spp. infections that will be highlighted. The panel will, also, include discussions on the applications of next generation sequencing (NGS), which have led to a better understanding of *Vibrio* strains that are more likely to cause human illnesses. How is this new information being incorporated into management strategies? With advancements in ecological forecasting abilities, what possibilities exist for mitigation of vibriosis? These topics and more will be discussed by federal, state, academic, and non-government organization (NGO) representatives to facilitate a comprehensive dialogue about changing the upward trend of vibriosis associated with seafood products.

RT4 Artisanal Food Processing and Food Safety

PANAGIOTIS LEKKAS: *University of Vermont, Burlington, VT, USA*
BARBARA INGHAM: *University of Wisconsin-Madison, Madison, WI, USA*
ANNA PORTO-FETT: *U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, USA*
JOSEPH CORBY: *Association of Food and Drug Officials, New York, NY, USA*

Artisanal food processing has become more popular over the past decade. Artisanal cheeses and meat products are, now, common gourmet food items. In 2000, the Association of Food and Drug Officials (AFDO) conducted a series of workshops across the United States for both retail meat processors and inspectors. One of the outcomes was a series of guidelines for processing that is now in the 6th edition. And yet, there are sporadic outbreaks

from retail and homemade artisanal products. This round table will discuss the problems, the issues, and potential solutions for the guidance and education of do-it-yourself (DIY) or chef curing in artisanal food processing.

RT5 What is IARC and CA Prop 65? What on Earth Do They Mean to Me and My Food Safety Program?

ERIC MITTENTHAL: *North American Meat Institute (NAMI), Washington, DC, USA*
GEORGE PUGH: *The Coca Cola Company, Atlanta, GA, USA*
CHRISTIE GRAY: *Decernis LLC, Rockville, MD, USA*
JAMES COUGHLIN: *Coughlin and Associates, Laguna Niguel, CA, USA*
LAURIE DOLAN: *U.S. Food and Drug Administration - HHS, College Park, MD, USA*

Riding a wave of high-tech and an overall statewide surge, California has surpassed France and Brazil to become the world's sixth largest economy. To participate in this growing economy is to strategically navigate the complex and cumbersome regulatory system in California; California Proposition 65 (CA Prop 65) is one example of the complex system. CA Prop 65 "requires the State to publish a *list of chemicals* known to cause cancer, birth defects, or other reproductive harm. This list, which must be updated at least once a year, has grown to include approximately 800 chemicals since it was first published in 1987." And many of those compounds are in food and beverages; they include certain approved ingredients, as well as well-known and safe food packaging compounds. Additionally, the International Agency for Research on Cancer (IARC) an arm of the World Health Organization, continues to evaluate the cancer hazards of various agents found in food, the environment, and industrial settings. Recently, IARC evaluated red meat and processed meat. California has, consistently, leveraged the IARC rulings to identify compounds to add to their growing list. So what does CA Prop 65 and IARC have to do with food safety? What do I need to know about them and my ability to produce and sell a safe product? Do these institutions impact public perception and food safety? These are just a sample list of questions slated to be explored during the roundtable discussion on IARC and Prop 65. The roundtable participants will provide a general overview of the program; provide specific nuances and insights to increase knowledge, as well as provide case study examples and approaches used to successfully navigate the complex system. Participants will, also, discuss their impact on the U.S. regulatory decision making process, as well as public perceptions about food safety.

RT6 Can Industry and Government Take Safe Food Handling and Preparation Risks Out of the Hands of the Consumer?

SANJAY GUMMALLA: *American Frozen Food Institute, McLean, VA, USA*
DONNA GARREN: *American Frozen Food Institute, McLean, VA, USA*
KELLY STEVENS: *General Mills, Minneapolis, MN, USA*
LONE JESPERSEN: *Cultivate, Hauterive, Switzerland, Switzerland*
KRISTINA BARLOW: *U.S. Department of Agriculture–FSIS, Washington, DC, USA*
TAMIKA SIMS: *IFIC, Washington, DC, USA*

Despite an uptick in outbreak reporting and increased public discussion regarding foodborne illnesses and recalls, consumers' risky practices continue to persist in the form of noncompliance. Consumers may perceive not ready-to-eat (NRTE) foods as having already been cooked in some way or posing no significant risk if consumed raw/undercooked and, thus, do not follow cooking instructions as directed. For this reason, foodborne outbreaks of commodities intended to be further processed by the consumer (i.e., cookie dough, frozen vegetables, and other NRTE foods) have occurred. By ignoring or unknowingly neglecting a kill step that could eliminate or reduce pathogens, consumers subject themselves to potentially avoidable risks of foodborne illness. While governmental agencies and food companies play a role in providing risk messages, such as safe cooking and handling instructions that are printed on labels, the responsibility for engaging and applying these messages falls upon the consumer. At what point does industry and government intervene with repetitive, risky practices implemented by consumers? New procedures, such as policy implementation or further processing of ingredients, can be an effective upstream preventative measure in reducing food safety risk; while a knowledgeable consumer who identifies possible risks is an effective downstream preventative measure. This roundtable will serve as a collaborative effort between industry, government, and consumer interest groups to identify points of disconnect in the food system. The objectives of this roundtable will be to (a) Discuss outbreaks related to continuous noncompliance by consumers; (b) Explore how procedures have evolved/can evolve to protect consumers from both a regulatory and industrial standpoint; (c) Highlight the current facets of food safety information distribution and acquisition between industry, government representatives, and consumers; and (d) Propose future steps in reducing the risk of potential outbreaks with foods that require further processing by consumers.

RT7 It's Going to Take a Village: Grower Perspectives on FSMA Implementation

BOB ZIEL: *J & J Family of Farms, Loxahatchee, FL, USA*
JANIE SIMMS HIPP: *Indigenous Food and Agriculture Initiative, Fayetteville, AR, USA*
CHELSEA MATZEN: *National Farmer's Union, Washington, DC, USA*
SAMIR ASSAR: *U.S. Food and Drug Administration, College Park, MD, USA*
ROBERT SAKATA: *Sakata Farms, Brighton, CO, USA*
ELIZABETH BIHN: *Produce Safety Alliance, Geneva, NY, USA*

Despite Good Agricultural Practices (GAPs) training and the development of food safety plans, many growers have concerns about the struggle to meet the new produce safety standards established by the Food Safety Modernization Act (FSMA), voluntary grower agreements, and buyers. One cause of concern is a lack of guidance regarding the implementation of FSMA, which creates unnecessary roadblocks to the implementation of these standards, and has legal and economic implications for growers. By bringing together a diverse group of grower representatives from multiple produce-growing regions and farm types/sizes, extension agents, and government stakeholders, this round table will provide a forum for discussing grower concerns about FSMA and conforming to these new standards.

This discussion will enable (i) growers and grower representatives to discuss roadblocks they are, currently, encountering as they adapt their practices to meet the new regulations (ii) extension agents and researchers to identify areas where improved communication is needed, and (iii) government stakeholders to identify areas where improved guidance is needed. The round table will foster discussion of these issues from the perspective of growers, and will facilitate substantive conversation between extension agents, grower representatives, and other stakeholders involved in the production of safe food. By providing a forum for government and academic stakeholders to engage with the grower community, this round table will further integrate the IAFP and grower communities. In doing so, it will increase IAFP members' understanding of grower needs, and, therefore, areas where more research is needed to directly benefit growers. The round table is a prime opportunity to involve developing food safety professionals interested in pre-harvest food safety by providing a forum to interact with the grower community. While there have been multiple round tables discussing challenges surrounding FSMA adoption, few have included small growers or emerging food safety professionals in the conversation.

RT8 International Strategies to Deliver Food Safety Education via the "Trusted Source": Health Professionals

JEFFREY LEJEUNE: *The Ohio State University, Wooster, OH, USA*
GLEE VAN LOON: *University of California-Davis, Health System, Sacramento, CA, USA*
ANTHONY FLOOD: *International Food Information Council, Washington, DC, USA*
ELLEN W. EVANS: *ZERO2FIVE Food Industry Centre, Cardiff, United Kingdom, United Kingdom*
SHAUNA HENLEY: *University of Maryland Extension, Baltimore County, Cockeysville, MD, USA*
YAOHUA FENG: *University of California-Davis, Davis, CA, USA*

Previous symposia have focused on food safety education for consumers, especially among high-risk populations. General consensus is that high-risk audiences would benefit from seeking advice from healthcare professionals, adequately trained in delivering targeted food safety information. However, not all healthcare professionals are aware of their patient's increased vulnerability to foodborne illness, nor do they consider themselves experts in food safety. Consumer food safety research suggests healthcare professionals are the most trusted and desired source for food safety information; however, such information has been found to be inconsistent. There is a need for communication between food safety experts and healthcare professionals. Consequently, the goal of this session is to facilitate a panel discussion among researchers, food safety educators and health professionals from the US and the UK to consider how best to develop an effective food safety strategy to enable healthcare professionals to deliver credible and reliable food safety information.

The internationally recognized panel includes three researchers, two educators, and one healthcare professional. They will consider the barriers and motivators that exist for healthcare professionals to deliver food safety advice to patients and how best to enable healthcare professionals to promote food-wellbeing through the delivery of targeted food safety and nutritional advice to individuals at an increased risk of foodborne illness. The need to change dietetic curriculum from trainee-dietitians having awareness of food safety legislation to focusing on delivery of food safety information, along with methods to enhance registered dietitians attitudes to consciously and proactively (rather than passively and reactively) deliver food safety information to patients, family-caregivers, and other healthcare professionals will be explored. This session will help set the stage for the development of a collaborative approach to improving the outputs of the domain and ultimately impact public health and start an international healthcare food safety education initiative.

RT9 Next Generation Whole Genome Sequencing in the Regulatory Arena: Nomenclature, Pipelines, Applications, and Collaboration

PETER GERNER-SMIDT: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
BILL KLIMKE: *NCBI, Washington, DC, USA*
ERIC BROWN: *U.S. Food and Drug Administration, College Park, MD, USA*
GLENN TILLMAN: *U.S. Department of Agriculture-FSIS-OPHS, Athens, GA, USA*

Worldwide public health organizations are moving into the world of Whole Genome Sequencing (WGS) as the standard for molecular characterization. This presents many advantages; however, there are still some issues to work through. This roundtable will explore the myriad of standardization issues, such as nomenclature (static versus dynamic, multi-locus, etc), pipelines, criteria, and future developments in platforms, metagenomics, and other topics we may not even be aware of yet. The round table panelists will discuss how their organizations are navigating these issues and the current status of WGS implementation.

RT10 FoodOmics: Stop Using a Steamroller to Crack a Nut!

ERIC BROWN: *U.S. Food and Drug Administration, College Park, MD, USA*
MIEKE UYTTENDAELE: *Ghent University, Ghent, Belgium, Belgium*
ROY BETTS: *Campden BRI, Gloucestershire, United Kingdom, United Kingdom*
KENDRA NIGHTINGALE: *Texas Tech University, Lubbock, TX, USA*
DOUGLAS MARSHALL: *Eurofins Scientific Inc., Fort Collins, CO, USA*
DANIEL SMIESZEK: *Nestlé, Dublin, OH, USA*

FoodOmic technologies have provided various new approaches to better study and understand food microbiology; all of them have highlighted the interactions between bioinformatics, transcriptomics, metagenomics, and metabolomics in order to study the microbiological continuum in various environments, including food products and food manufacturing facilities. The food industry may now feel over knee-deep with these omic communications, and may question their use related to food safety and quality issues. How can we apply them? What do they mean? Finally, are these omic-based analyses just tools like a novel pocketknife for microbiologist? The round table will address some basic questions and considerations and will offer pertinent debates around the following points: How to select the fit for purpose tool and avoid overrunning with a deluge of data and information? Stop using a steamroller to crack a nut and use the appropriate tool to go straight to result! What are the expected outcomes in order to improve microbial risk-benefit assessment and help the decision makers? But never forget that false positive rate, time to result, and handling time are the basic expectations in routine testing. How to control, define, and standardize these technologies to make sure they will benefit most food business operators and ensure a clearer understanding of the applications in food production and product development? How to stay up to date while these expected, disruptive technologies change laboratory facilities and analyst's profiles? Is the food industry going to lose its soul by generating so many data sets? Are we going to create more issues for the food industry or contribute a better understanding on how to control and possibly fix these issues?

RT11 National and Regional FSMA Training Centers: Application of Lessons Learned

ELIZABETH NEWBOLD: *University of Vermont, Bennington, VT, USA*
ANGELA SHAW: *Iowa State University, Ames, IA, USA*
ROBERT MCGORRIN: *Oregon State University, Coralville, OR, USA*
MICHELLE D. DANYLUK: *University of Florida, Lake Alfred, FL, USA*
DAVID READ: *IFPTI, Battle Creek, MI, USA*
ELIZABETH BIHN: *Cornell University, Geneva, NY, USA*

The overall theme of this roundtable is: "How to Assist Fruit and Vegetable Growers and Processors Comply with the FSMA Produce Safety and Preventive Controls Human for Food Rules". The U.S. Food and Drug Administration (FDA), along with the U.S. Department of Agriculture's National Institute of Food and Agriculture, recently, funded a national and four regional centers, through a food safety training, education, extension, outreach, and technical assistance grant program, as a mechanism to support stakeholder education. The centers are funded to provide training, education and technical assistance, that is consistent with the standards being established under the Food Safety Modernization Act (FSMA), to owners and operators of small and medium-sized farms, beginning farmers, socially disadvantaged farmers, small food processors, and small fruit and vegetables merchant wholesalers affected by FSMA requirements. Each regional center has conducted needs assessments on what the training needs are for growers and processors and will manage and coordinate regionally appropriate assistance programs for these stakeholders.

Roundtable panelists will focus on true educational needs of the target growers and processors in the U.S. and how educators are beginning to meet these needs. They will also share early evaluation data from center activities and new educational materials that have been developed in response to needs. Participants will leave session knowing the research gaps and what extension curriculum tools are available for growers and processors wanting to be in compliant with FSMA.

RT12 The Devil is in the Details: Experiences with Early Implementation of the FSMA Produce Safety Rule and Efforts to Fill the Information Gaps

BOB EHART: *National Association of State Departments of Agriculture, Arlington, VA, USA*
JENNIFER MCENTIRE: *United Fresh, Washington, DC, USA*
JAMES RUSHING: *JIFSAN-University of Maryland, College Park, MD, USA*
TREVOR SUSLOW: *University of California-Davis, Davis, CA, USA*
MICHELLE D. DANYLUK: *University of Florida, Lake Alfred, FL, USA*
DON STOECKEL: *Cornell, Geneva, NY, USA*

FDA's Produce Safety Rule creates, for the first time, federal requirements for the on-farm production and handling of produce (new 21 CFR Part 112). The compliance dates for the largest farms will be January, 2018. FDA estimates that over 37,000 U.S. and foreign farms exporting produce to the U.S. will be covered by the rule. More farms may be impacted, either through voluntary adoption or through buyer requirements.

In some places, rule requirements are very specific. In other places, FDA focused on the basic objectives or "end points" rather than being prescriptive about what approach needs to be used. This flexibility recognizes the diversity of practices and conditions related to produce production. In some cases, the science is still evolving. However, even where the basic scientific principles are well established, there may not be sufficiently specific data to provide practical guidance about how to comply or how practices might be implemented on very diverse operations. Identifying and filing information, education, outreach, guidance, and research needs to facilitate compliance with this new regulation will be a massive effort involving a host of regulatory and technical assistance and training groups, many of them newly created for this purpose.

Roundtable panelists will share their experience in early implementation, such as impressions from the cooperative FDA/NASDA on-farm readiness reviews; questions raised through technical assistance networks (TANs), national and regional centers, and at alliance trainings. This roundtable will begin a conversation about (i) the current state of on-farm readiness; (ii) challenges to implementation, including understanding of FDA's expectations for compliance with rule requirements and access to sufficient tools and technical resources for implementation; and (iii) how the groups represented by the panel might work, independently and in collaboration, to assist farms in implementing and achieving science-based and measurable food safety objectives.

RT13 Variations on a Theme: The Basis and Consequences of Inconsistent *Listeria* spp. Standards in Global Regulation

TAMIKA SIMS: *IFIC, Washington, DC, USA*
JANE VAN DOREN: *U.S. Food and Drug Administration, College Park, MD, USA*
MARTA HUGAS: *European Food Safety Authority, Parma, Italy, Italy*
KRIS DE SMET: *European Commission, Brussels, Belgium, Belgium*
JEFFREY FARBER: *University of Guelph, CRIFS, Department of Food Science, Guelph, ON, Canada, Canada*
MICKEY PARISH: *U.S. Food and Drug Administration, College Park, MD, USA*

CODEX Alimentarius has been advocating that food regulations be developed using sound science and risk assessments as input into risk management decision making. However, this may not always lead to consistent regulations. A case in point are *Listeria* spp. regulations, which differ substantially, internationally, as a result of differences in risk management decisions taken in different jurisdictions. Does that mean that there are major differences in the sound science and risk assessment base? Or, are risk managers taking into account those insights in different ways? Recent developments in risk assessment have added to past scientific and risk assessment insights. Will these insights trigger risk managers to reconsider their regulations? In any case, inconsistencies in regulatory standards for *Listeria* spp. or other pathogens cause confusion with stakeholders, such as consumers and industry, when risks and risk management interventions are communicated.

This session will explore recent developments in risk assessment, risk management, and risk communication to better understand the basis of inconsistencies in risk management interventions and how risk communication addresses these inconsistencies at the international level. An update on risk assessment from the USA will be followed by risk management views from different jurisdictions (USA, Canada, and EU) and a discussion on challenges communicating conflicts in risk management views to stakeholders (from USA and EU).

RT14 Hog Slaughter Modernization and *Salmonella* Performance Standards: Should Pork be Treated the Same as Poultry?

WILLIAM SHAW: *U.S. Department of Agriculture-FSIS-OPPD, Washington, DC, USA*
DEIRDRE SCHLUNEGGER: *STOP Foodborne Illness, Chicago, IL, USA*
MICHAEL BRADLEY: *Smithfield, Clinton, NC, USA*
KATIEROSE MCCULLOUGH: *North American Meat Institute, Washington, DC, USA*
MARTIN APPELT: *Canadian Food Inspection Agency, Ottawa, ON, Canada, Canada*

In the "*Pathogen Reduction; Hazard Analysis and Critical Control Point Systems*" (PR/HACCP) final rule (61FR 38806; July 25, 1996), USDA-FSIS announced plans to evaluate new models for testing meat and poultry products. One of these models is the HACCP-Based Inspection Models Project (HIMP), in which packing plants take greater responsibility for carcass inspection. In the intervening period, FSIS has conducted baseline studies leading to performance standards for *Salmonella* and *Campylobacter* in poultry; and in 2015, began implementation of the Poultry Inspection Modernization Rule.

For pork, FSIS has announced their intention to modernize hog slaughter regulations, including establishment of *Salmonella* performance standards, which, now, only apply to market hogs. FSIS has conducted comparisons of the five HIMP pork pilot project producers and non-HIMP slaughter facilities and is using the data to determine if new performance standards for pork are warranted. Agencies within other governments, notably the Canadian Food Inspection Agency (CFIA), are investigating ways to modernize and augment their pork safety inspection and testing programs. Some industry stakeholders are calling for delay of U.S. hog slaughter modernization until data adequately demonstrate that it will not adversely affect public health, worker safety, or animal welfare. Others propose that alternative approaches can enhance safety of pork products without the need for additional performance standards. This roundtable will address the regulatory, industry, consumer, and stakeholder perspectives on new efforts to improve pork safety and quality.

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Symposium Series on Food Microbiology

Sponsored by the
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ILSI Europe's Microbiological Food Safety Task Force

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The International Association for Food Protection (IAFP) is a non-profit association whose mission is to provide food safety professionals worldwide with a forum to exchange information on protecting the food supply.

The North American Branch of the International Life Sciences Institute (ILSI North America) is a non-profit organization based in Washington, D.C., that plays an important role in identifying and addressing scientific questions on nutritional quality and food safety.

IAFP and ILSI North America have been collaborating since 1993 to bring you the Symposium Series on Food Microbiology.

S23 How to Exploit Omics Data on Pathogen Behavior in Microbiological Risk Assessment: An Update on the Current Research

Following the technological advancements in the field of the nucleic acids sequencing and the possibility to obtain a large number of sequences (millions) from the microorganisms present in a single sample without the need for their cultivation, new opportunities have become available in terms of data production and exploitation in the field of microbiological risk assessment (MRA). More specifically, the behavior of foodborne pathogens, deciphered with transcriptomic, proteomic, and/or metabolomics techniques, during the whole food chain and in response to specific stresses, can now be studied. The current challenge scientists are facing is the integration of such data into risk assessment schemes.

In 2016, at the IAFP European Symposium in Athens, the workshop, "Next Generation MRA (Microbiological Risk Assessment) - Integration of Omics Data into Assessment" was co-organized by ILSI Europe, IAFP, and ICFMH. During the workshop, four breakout groups (epidemiology, metagenomics, exposure assessment, and hazard characterization) brain-stormed and produced a common strategy to go beyond the current knowledge. In this symposium we will present the main outcomes of the workshop, including the points of view of the academia and the industry, and we will advance the discussion related to how to best use omics data in MRA.

The Use of Metagenomics in Quantitative Microbiological Risk Assessment (QMRA)

KALLIOPI RANTSIOU, *University of Turin-DISAFA, Turin, Italy*

The application of next generation sequencing techniques in food samples offers the potential to investigate the microbial composition and functions in unprecedented depth and in high throughput fashion. Metagenetics, or amplicon sequencing, is an approach that is 'taxonomy oriented', gives a detailed view of the composition of a system and has been applied to describe the microbial ecology of foods and food-related ecosystems. Metagenomics is a 'function' oriented approach and has, as of yet, been less exploited in food microbiology. Both approaches can enrich our understanding regarding interactions between biotic and abiotic factors but also within the microbial community of any given ecosystem. Understanding such interactions and the behavior of microorganisms is important in the process of assessing biological risks in foods. For example, associations between specific microbial communities and the presence of a foodborne pathogen can be unraveled by the culture-independent analysis of large sets of samples. Further, such association may lead to the identification of community fingerprints to trace foodborne pathogens at species or even strain level and their transmission through the food chain. It is envisioned that risk assessment will gradually integrate information that concern the behavior of microorganisms, assembled from 'omics' data, and shift from the taxonomic definition of the biological hazards. Improvement of the resolution of the metagenomics data would allow us to observe and study the foodborne pathogens in their environment even when they are not prevailing components of the microbial community.

The Use of Omics in Exposure Assessment

HEIDY DEN BESTEN, *Wageningen University, Wageningen, Netherlands*

Exposure assessment plays a central role in a microbiological risk assessment as it provides an estimation of both the likelihood and the level of a microbial hazard in a specified consumer portion of food, taking microbial behaviour into account. To date, mostly phenotypic data have been used in exposure assessment. This presentation will illustrate how mechanistic cellular information obtained through omics techniques could make a difference: (i) in understanding the dynamics of pathogens in a complex food eco-system, and, (ii) in predicting pathogen behaviour variability. Advancements in the research activities of these two domains will be presented and discussed, through examples, with a special focus on industrial applications.

The Use of Omics in Hazard Characterization

TREVOR PHISTER, *PepsiCo, Leicester, United Kingdom*

Omics technologies such as Whole Genome Sequencing (WGS) have impacted food safety through the incorporation of WGS into epidemiological investigations. While there are still many questions around the use of WGS in source identification, the technology has already been adopted by a number of public health agencies. Academics, industry and regulatory bodies, however, have only just begun to explore the integration of omics data into microbial risk assessment (MRA).

In this presentation, we will discuss the issues and challenges in using omics data in hazard characterisation. The data may aid in decreasing the variability and uncertainty present in this stage. The current research suggests omics may be integrated in a number of ways from defining the differences in virulence between bacterial strains to the identification of biomarkers that may suggest increased virulence of a pathogen or susceptibility of a host. Omics use in MRA is just beginning and as it has with epidemiology it is sure to have a big impact on how we characterise hazards in our food supply.

S24 Battling Bad Bugs: Biological Approaches to Control Pathogens

The food industry often relies on physical and chemical interventions to control foodborne pathogens. This session is intended to discuss biological approaches to control foodborne pathogens throughout the food continuum. Topics to be covered include the use of (i) biocontrol of *Listeria monocytogenes* and *Salmonella enterica* Serovar Poona on fresh-cut apples with naturally occurring bacterial and yeast antagonists, (ii) probiotics to reduce the load of foodborne pathogens that enter the human food chain and pathogen populations on food and in food processing environments and (iii) bacteriophage to combat bacterial foodborne pathogens. This session will, also, include discussions on consumer perception and acceptance of these biological approaches to control foodborne pathogens.

Biocontrol of the Foodborne Pathogens *Listeria monocytogenes* and *Salmonella enterica* Serovar Poona on Fresh-cut Apples with Naturally Occurring Bacterial and Yeast Antagonists

WOJCIECH JANISIEWICZ, *U.S. Department of Agriculture–ARS, Kearneysville, WV*

Application of Probiotics to Control Foodborne Pathogens from Farm to Fork

MINDY BRASHEARS, *Texas Tech University, Lubbock, TX*

Application of Bacteriophage to Control Foodborne Pathogens in the Food Processing Environment and in Ready-to-Eat Foods

SAM ALCAINE, *Cornell University, Ithaca, NY*

S34 Managing Risk in a Zero Tolerance World

It is accepted that there is risk in everything and the discipline of risk assessment focuses on minimizing risk to an acceptable level. This seems to be in conflict with the concept of zero tolerance, which is not completely codified in regulation; and yet, does seem to exist in actual food industry practice. Does the current interpretation of FSMA rules and regulatory application of new technologies in outbreak investigation drive the expectation of a zero-risk environment? What should risk assessment and food safety management look like in this changing landscape? How can risk be managed to allow suitable options for consumers and food manufacturers? The perception and understanding of risk necessitates a conversation between regulators, consumers, and the food industry.

The Changing Landscape: Implications of New Regulations on Risk Assessment

DON ZINK, *IEH Laboratories & Consulting Group, Herndon, VA*

The International Dynamic of Risk Assessment

ROBERT BUCHANAN, *University of Maryland, College Park, MD*

How is Whole Genome Sequencing Impacting Assessments of Risk and Setting of Standards?

LUCA COCOLIN, *University of Torino-DISAFA, Grugliasco, Italy*

Setting Risk-based Performance Standards

ROY BETTS, *Campden BRI, Gloucestershire, United Kingdom*

Risk Management: Strategies and Challenges in a Zero Risk Environment

TIMOTHY JACKSON, *Nestle USA, North America, Glendale, CA*

Consumer Perceptions of Risk and How It Influences Their Choices

WILLIAM HALLMAN, *Rutgers University, New Brunswick, NJ*

S48 Foodborne Viruses: Detection, Risk Assessment, and Control Options in Food Processing

Foodborne viruses are recognized among the top rated food safety priorities in a very recent report of risk assessment experts on the identification of food safety priorities using the Delphi technique (Rowe and Bolger, 2016) and have become, over the past few years, a greater concern to the food industry. All parties agree that control measures for viruses throughout the food chain are required; however, much still needs to be understood with regard to the effectiveness of these controls and how to properly validate their performance; whether it is the personal hygiene of food handlers, the effects of processing on foods at risk, or the interpret and action on a positive test result in a virus testing program (EFSA, 2011 and FSA, 2015).

In this session, we will present the current work of an ILSI Europe expert group that provides a description of foodborne viruses, their characteristics, and responses to stress. There will, also, be a critical discussion on the technologies developed for their detection and control and the way forward on the applications for science and industry. The recommendations in this review will allow industry to perform effective control options for viruses in food processing. We will present the current state of the science in epidemiology, public health burden, risk assessment, and management options for viruses in food processing environments and will draw practical conclusions.

Pros and Cons of Methods of Detection for Viruses in Foods

ALBERT BOSCH, *University of Barcelona, Barcelona, Spain*

Enteric viruses, particularly human noroviruses (NoV) are the most common cause of food borne disease, and a significant contributor to global disease burden. Viruses enter the food supply across the farm-to-fork chain by exposure to contaminated waters, surfaces, and/or human hands. Unlike bacterial pathogens, for which there are widely used validated detection technologies, virus detection methods are less well developed. There are many reasons for this which, when taken together, necessitate processing food or environmental samples for virus concentration and purification prior to application of detection methods. These detection methods can be cumbersome, expensive, inefficient, and fraught with complications, including the inability to discriminate infectious from non-infectious viruses. The purpose of this presentation is to discuss the outcomes arising from ILSI Europe expert Group recent work, highlight recent advances in viral detection for human NoV, Hepatitis A and emerging viruses such as Hepatitis E, the pros and cons of various detection methods and its applicability to foods and environmental samples, recent developments in food virology arena and how these methods can be used to address real-world issues.

Translating Risk Assessment of Viruses in Foods into Practice

TREVOR PHISTER, *PepsiCo, Leicester, United Kingdom*

Viruses are the most frequent cause of foodborne illness worldwide and a major contributor to the global foodborne disease burden. To assess risks associated with viruses and other hazards in the food chain and set appropriate control measures, the use of risk assessment techniques has been suggested by international bodies and increasingly accepted by governments around the world as a basis for national legislation in relation to food safety. There are two main approaches in performing a microbiological risk assessment (MRA), an epidemiological approach (top-down approach) starting from data on illness and moving towards the hazard in the product and a food chain approach (bottom-up approach) starting from the hazard in the product and moving towards an estimate of the probability of illness.

This presentation aims to give a general introduction into the use of MRA by both industry and governments as a tool for quantifying the risk of foodborne illness due to viruses and to discuss bottlenecks and differences in available methodologies (top-down and bottom-up), providing examples from recent literature. A special focus will be given into translating the results of MRA into practical interventions for the protection of public health.

Effect of Processing Technologies to Control Viruses in Foods

ALVIN LEE, *Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, IL*

Traditionally, processing technologies rely on the control of bacterial contaminants as a measure of their effectiveness. However, various studies have shown that some foodborne viruses are more resistant than vegetative bacteria to certain control mechanisms and thus may not be inactivated at the same rate as bacteria. In addition, as the food industry increasingly moves towards milder thermal processes, as well as the use of non-thermal technologies, the likelihood of viruses surviving such treatments increases. Therefore, validations of control strategies also need documented scientific evidence to demonstrate the effectiveness of control measures for reducing or eliminating viruses from foods. Validation approaches are hampered by the difficulty in cultivating enteric viruses. The replication assay recently developed for certain human norovirus strains should in the future allow evaluation of control strategies for these viruses. However, at present, the most common approach has been to use cultivable surrogate viruses such as Feline Calicivirus, Murine norovirus, Tulane virus and bacteriophages such as MS2. This presentation will give an overview on the efficiency of intrinsic and extrinsic factors of foods and various processing technologies, both traditional thermal and non-thermal technologies, and chemical based technologies to inactivate enteric viruses in foods. There are difficulties in comparing inactivation studies as numerous factors such as surrogate choice and its preparation, treatment time, inoculation methods and time allowed for inoculum to attach to the product could have a significant impact on the reduction observed. A standardised method for evaluating decontamination strategies for foods would be very useful for the food industry and future research needs to develop such as guidance will be discussed.

S56 Chemical and Microbial Risk Assessment: Similarities and Differences

In food safety, risk assessment is the scientific evaluation of known or potential health effects resulting from human exposure to chemicals or microorganisms. Exposure to pathogenic microorganisms is different from exposure to chemicals. Microorganisms differ from chemicals, as they can grow or die and the level of exposure to microorganisms can change, dramatically, in a short period of time. The speakers in this session will discuss the similarities and differences between chemical and microbial risk assessment; risk-risk and risk-benefit analysis. Some case studies will, also, be presented.

Chemical Risk Assessments and Their Uses in Decision Making

JOSEPH RODRICKS, *Ramboll Environ, Arlington, VA*

The presentation will describe the types of evidence used to conduct chemical risk assessments. Evidence regarding three questions will be discussed:1) that used to identify the chemical's important toxic hazards ; 2) that used to identify the quantitative relationship between the risk of toxicity and dose; and 3) that pertaining to the exposures to the chemical experienced by the populations of interest. Integrating results from these three analytic steps will answer the question of the nature and magnitude of health risk experienced by the populations of interest. It also allows understanding of whether and to what extent controls on exposure are needed to avoid excessive risks.

The major issues to be addressed, which are also the most significant in highlighting the differences between chemical and microbial assessments, concern the many scientific uncertainties that arise in the evidence, and in the need to extrapolate from the populations and conditions under which evidence has been collected to those conditions and populations of interest for risk assessment . Included are those that arise because of the need in most cases to rely upon animal studies for hazard and dose-risk information, those related to the need to extrapolate from high dose findings to relatively low dose human exposures, and the uncertainties associated with variabilities in response within and across human populations. These uncertainties require the use of incompletely verified assumptions in virtually all risk assessments. Standardized assumptions are often used, but can be replaced in specific cases in which scientific studies have identified approaches having reduced uncertainty. Key differences between microbial and chemical risk assessments will be summarized.

Quantitative Microbiological Risk Assessment: Dealing with Biological Diversity

ROBERT BUCHANAN, *University of Maryland, Department of Nutrition and Food Science and Center for Food Safety and Security Systems, College Park, MD*

During the past 20 years, quantitative microbiological risk assessment (QRMA) for evaluating food safety issues has evolved from an object of academic curiosity to an established risk analysis sub-discipline. QMRA techniques are increasingly used by both industry and regulatory agencies to inform priority setting, the relative effectiveness of potential risk management options, the development of integrated food safety systems, and the impact of pathogenic/toxigenic microorganism of high-risk segments of the consuming public. The development of QMRA methods has been strongly influenced by earlier work in chemical risk assessments, as reflected in the adoption of the four component approach to risk assessments, i.e., hazard identification, hazard characterization, exposure assessment, and risk characterization. However, unlike many chemical risk assessments, the dramatic and often rapid changes in the levels of a pathogenic microorganism that can occur along a farm-to-fork food chain had to be a major focus of QRMA development. For example, a single cell of *Salmonella* can increase to billions per gram overnight, and then plummet back to a single cell after a 5-minute heating on the stove. Likewise, the susceptibility of the human population to pathogenic microorganism routinely varies from highly sensitive, immunocompromised individuals to others who are totally immune due prior exposures or vaccines. Likewise, the relative pathogenicity of individual species can vary substantially depending on the virulence determinants individual strains have acquired, and the mechanisms of disease among the various classes of foodborne pathogenic microorganisms. For example, dose-response relations for infectious and toxico-infectious agents are typically best described with non-threshold models, whereas toxigenic agents are better described with threshold models. Advances in QMRA helping find practical means for decreasing uncertainty by better understanding and describing the impact biodiversity on risk. This focus is also helping find new methods for addressing biodiversity issues associated with chemical and environmental risk assessments, particularly for carcinogens and mutagens.

Safety and Risk Assessments: Fit for Purpose

JANE VAN DOREN, *U.S. Food and Drug Administration, College Park, MD*

FDA conducts safety and risk assessments to inform decisions about the products we regulate. The specific approach taken and methods depend upon the nature of the question these assessments are intended to answer. This presentation will describe the use of FDA tools including FDA-iRISK and will also examine the decisional context that guides when a safety assessment or a risk assessment approach is needed. Case studies will illustrate the value of using probabilistic approaches that take into account variability in parameter values and quantify uncertainty for both microbial and chemical hazards in food

D1 A Debate: Current Perspectives in Food Safety

This interactive roundtable is intended to engender lively discussion of important food safety topics. It is assumed audience participants will have a basic understanding of the unresolved issues surrounding the topics to be discussed in the symposium. The session will cover three topics: "The good, the bad, and the ugly foods: Should we encourage the consumption of "ugly" and expired foods?"; "Who's to blame? Do consumers own a piece of food safety?"; and, "Which is the real 'obesogen'—pizza or the pizza box?" Each topic will include a 7-minute presentation in support of (Yes, Pizza) followed by a 7-minute presentation in opposition of (No, Pizza Box) the proposed topic question. Each speaker will have 3 minutes for extemporaneous rebuttals. A 6-minute question/answer session will then follow to allow for audience participation. We will have electronic polling of the audience to allow for a Yes/No vote on each topic question prior to and following the discussion to evaluate whether people's views have been changed by the presentations..

The Good, the Bad, and the Ugly Foods: Should We Encourage the Consumption of Ugly and Expired Foods?

SARAH CAHILL, *Food and Agriculture Organization of the United Nations*

ROBERT TAUXE, *Centers for Disease Control and Prevention*

Who's to Blame? Do Consumers Own a Piece of Food Safety?

SARAH BREW, *Faegre Baker Daniels LLP*

BILL MARLER, *Marler Clark, The Food Safety Law Firm*

Which is the Real Obesogen? The Pizza or the Pizza Box?

RUTH KAVA, *American Council on Science and Health*

Technical Abstracts

T1-01 Thermal Tolerance of Foodborne Pathogens on Inoculated Pistachios

MAHTA MOUSSAVI, Christopher Theofel and Linda J. Harris
University of California-Davis, Davis, CA

Introduction: Several recent outbreaks and recalls have been associated with pistachios contaminated with *Salmonella*. Thermal treatments are the main strategy used by the pistachio industry to control this organism.

Purpose: This study identified an appropriate target pathogen and surrogate organism for validation of thermal processes applied to pistachios.

Methods: Inshell pistachios were inoculated at nine log CFU/g with nonpathogenic strain *Enterococcus faecium* NRRL B-2354, *Salmonella* Enteritidis PT 30, or relevant individual strains (five each) of *Salmonella* spp., *Escherichia coli* O157:H7, or *Listeria monocytogenes*; pistachios were dried for 72 h, and equilibrated to a mean moisture content of 3.6±0.28% (water activity 0.37±0.03). Thermal tolerance of each strain was compared by exposing inoculated pistachios (n=3 to 9) to hot oil (121°C/1 min), hot water (80°C/1 min), or dry heat (138°C/15 min). Survivor curves in hot oil or hot water (0.5 to 6 min, n=6) were developed for the most resistant strains. Survivors were enumerated by plating on tryptic soy agar and appropriate selective media.

Results: In most cases, after exposure to heat treatments, reductions in populations of individual strains of *Salmonella* spp., *E. coli* O157:H7, or *L. monocytogenes* were significantly greater (P<0.05) or not significantly different than reductions of *E. faecium* and *Salmonella* Enteritidis. Survivor curves for the more resistant *E. coli* O157:H7 and *L. monocytogenes* strains (isolated from walnuts and onions, respectively) in hot water were not significantly different than those for *E. faecium* and *Salmonella* Enteritidis. In hot oil, significantly greater reductions of *E. coli* and *L. monocytogenes* were observed after three or two minutes of exposure, respectively.

Significance: These data provided further evidence that *Salmonella* spp. and *E. faecium* NRRL B-2354 are the appropriate target pathogen and an appropriate surrogate, respectively, for validating pistachio thermal processes.

T1-02 Colonization and Internalization of *Salmonella enterica* in Cucumber Plants

KELLIE P. BURRIS[1], Otto Simmons[1], Hannah M. Webb[1], Lee-Ann Jaykus[1], Jie Zheng[2], Elizabeth Reed[2], Christina Ferreira[2], Eric Brown[2] and Rebecca L. Bell[2]
[1]North Carolina State University, Raleigh, NC, [2]U.S. Food and Drug Administration, College Park, MD

Introduction: Consumption of fresh produce has been linked to numerous foodborne outbreaks involving *Salmonella enterica*. A recent outbreak of *Salmonella* Poona was associated with consumption of slicer cucumbers.

Purpose: This study examined the preharvest risks of cucumber when exposed to *Salmonella* spp. on blossoms and in soil.

Methods: Cucumber plants (*Cucumis sativus* var. *sativus*) from two cultivars, Puccini (pickle) and Thunder (slicer), were grown from commercial seed. Plants were maintained in the NCSU BSL-3 phytotron greenhouse. *Salmonella* (a cocktail of serovars Javiana, Montevideo, Newport, Poona, and Typhimurium) contamination was introduced via blossoms or soil at six log CFU/blossom or eight log CFU/root zone. Cucumbers were analyzed for *Salmonella* spp. by enrichment in accordance with modified FDA-BAM methods. Five randomly chosen colonies from each *Salmonella* spp.-positive sample were serotyped using the Agilent 2100 bioanalyzer following multiplex PCR. Data were analyzed for prevalence of contamination, serovar predominance in fruit and stems, and differences between cucumber cultivars.

Results: Of the cucumber fruit harvested from *Salmonella* serovar-inoculated blossoms (n=58), 83% (48 of 58) were contaminated and 67% (39 of 58) had *Salmonella* spp. internalized into the fruit; of those positive for *Salmonella* spp., greater than 70% (15 of 210) were identified as *Salmonella* Poona. Prevalence of contamination was equivalent when comparing cultivars (pickle vs. slicer) and location of contamination (outer vs. inner). When soil was inoculated at the base of 20 day-old plants, only 8% (10 of 120) of the plants were shown to translocate *Salmonella* spp. to the lower stem after seven dpi; of those positive, serovars Javiana, Montevideo, Newport and Poona each were identified at 20 to 30% (n=132).

Significance: These results identified blossoms as a route by which *Salmonella* spp. are internalized at a high percentage into cucumbers. *Salmonella* Poona, the same strain isolated from the 2015 outbreak of cucumbers imported from Mexico, was shown adapted to the blossom niche.

T1-03 Assessment of Zoonotic Risks in Aquaponic Lettuce Production: A Prototype for Experimental Greenhouse Trials

Elizabeth Antaki[1], Geoffrey Mangalam[1], Peiman Aminabadi[1], Fernanda de Alexandre Sebastião[2], Esteban Soto[2], Beatriz Martínez López[2], Fred Conte[2], Sarah Taber[3] and **MICHELE JAY-RUSSELL**[4]
[1]Western Center for Food Safety, University of California-Davis, Davis, CA, [2]University of California-Davis, Davis, CA, [3]Boto Waterworks, LLC, Gainesville, FL, [4]University of California-Davis, Western Center for Food Safety, Davis, CA

Introduction: Aquaponics is the integration of aquaculture and hydroponics that is now being used as a model for sustainable food production. Because fresh vegetables are usually consumed raw, especially leafy greens, there are concerns about food safety and zoonotic risks from fish waste for this emerging industry.

Purpose: The purpose of this study was to develop an experimental recirculating aquaponic system (RAS) prototype and to then investigate persistence and transfer via root uptake of an attenuated *Salmonella* strain in a RAS used for leafy green production in order to gain the knowledge of good agricultural practices (GAPs) specific for aquaponic practitioners to reduce the potential for foodborne illnesses due to product contamination.

Methods: Initially, the lethal and infective dose of *Salmonella enterica* serovar Typhimurium (aPTVS177) strain to naive tilapia (*Oreochromis* spp.) fingerlings was determined by intragastric challenge. Then using two nonlethal doses (10^5 and 10^9CFU), a second group of fish was challenged and used in the laboratory controlled RAS utilizing hydroponic lettuce. *Salmonella* was quantified in the system components (tanks, tubing, plant bed substrate), fish waste, and lettuce plants (roots and plants) using microbiological and molecular analysis.

Results: Only the three highest inoculums (10^8 to 10^{10} CFU) yielded positive isolation of *Salmonella* from fish. In the second challenge, at least one of the RAS holding *Salmonella*-infected fish was positive for the presence of *Salmonella* at the tested time points. On day 42, two fish from one RAS, inoculated with a high dose (10^9 CFU), had positive stomach and intestinal tissues with concentrations ranging from 0.36 to 160 MPN/g of tissue (MPN; most probable number). None of the plants or other tank system components were positive.

Significance: This study described a potential system to investigate foodborne diseases in a RAS and identified potential strategies to reduce the risk of bacterial contamination if exposed to contaminated fish waste, leading to the foundation of GAPs.

T1-04 Dynamic Changes in Water Quality and Microbial Survival during Commercial Fresh-cut Produce Wash Operation

YAGUANG LUO[1], Bin Zhou[1], Boce Zhang[1], Xiangwu Nou[1], Sam Van Haute[2], Ellen Turner[1], Zi Teng[2], Qin Wang[2] and Patricia Millner[1]
[1]U.S. Department of Agriculture–ARS, Beltsville, MD, [2]University of Maryland, College Park, MD

Introduction: Determining minimal, effective free chlorine (FC) concentration for preventing pathogen survival and cross-contamination is critical for developing science- and risk-based food safety practices.

Purpose: This study investigated the correlation between dynamic FC concentrations and bacterial survival under commercial fresh-cut produce wash operations.

Methods: Chopped romaine lettuce, shredded iceberg lettuce, and diced cabbage were washed in a two-flume system under typical commercial fresh-cut processing conditions. Targeted FC concentrations and pH levels were maintained using sodium hypochlorite and a phosphoric acid-based solution, respectively, via an automated control system. Wash water was sampled every 30 minutes and assayed for chemical oxygen demand (COD), turbidity, total dissolved solids (TDS), and FC. Bacterial survival was determined by plating aliquots of neutralized water samples on APC Petrifilms™.

Results: Significant increases in COD, turbidity, and TDS were observed over time, with more rapid increases during wash of diced cabbage. Combined chlorine concentration increased consistently over time; FC concentration fluctuated, as impacted by the rate of chlorine replenishment, cut product loading, and water replenishment. Bacterial survival in wash water correlated with FC concentration. With FC below approximately 10 mg/liter, increasing FC concentration resulted in sharp declines in the frequency and population of surviving bacteria detected. Further increases in FC concentration resulted in most total aerobic bacterial count being reduced to below the detection limit (50 CFU/100 ml); there were a few sporadic positive samples with low cell counts.

Significance: Results showed that maintaining at least 10 mg/liter FC in wash water contributes significantly to reduced survival of culturable aerobic bacteria. Tests with culturable, indigenous aerobic bacteria in produce wash conditions are valuable for commercial operations, since inoculating produce with human pathogens or even non-pathogenic surrogates during commercial operations is not feasible given the inherent food safety risks.

T1-05 Efficacy of Wash Water Disinfectants in Reducing Water-to-Mango Contamination by *Salmonella* under Simulated Mango Packing House Operations

ELZA NEELIMA MATHEW, Muhammed Shafeekh Muyyarikkandy and Mary Anne Amalaradjou
University of Connecticut, Storrs, CT

❖ ***Developing Scientist Competitor***

Introduction: Salmonellosis associated with consumption of mangoes has been traced back to the use of contaminated wash water. This highlights the critical role of wash water disinfection in the quality and safety of mango processing. While investigations on the efficacy of disinfectants to reduce pathogens on other fruits have been performed, no studies have been conducted on mangoes.

Purpose: This study evaluated the efficacy of chlorine (200 ppm), peracetic acid (80 ppm), and chlorine dioxide (5 ppm) for inactivating *Salmonella* on mangoes and in wash water under simulated mango packing house conditions.

Methods: Nalidixic-acid resistant strains of *Salmonella* Montevideo, *Salmonella* Newport, *Salmonella* Baildon, *Salmonella* Braenderup, and *Salmonella* Poona were used in this study. Disinfectants were added to inoculated wash water (~ seven log CFU/ml). Mangoes (var. Ataulfo and Tommy Atkins) were washed under simulated dump tank wash (24°C for 2 min), hot water treatment (46°C for 75 and 110 min), and hydrocooling conditions (21°C for 30 min). Wash water and mangoes were collected at different times for microbiological analysis. Additionally, pH, temperature, and disinfectant concentration of wash water were monitored throughout the study.

Results: Chlorine (200 ppm) and PAA (80 ppm) were effective in completely inactivating *Salmonella* populations on mangoes and in wash water during dump tank wash, hot water treatment, and hydrocooling in the presence and absence of organic matter. However, mango and water samples were positive (<0.9 CFU/mango or wash water sample) for *Salmonella* following treatment with chlorine dioxide, which could be attributed to the sharp drop in its residual concentration upon addition to water.

Significance: This study showed that commonly used disinfectants, chlorine and PAA were effective in controlling *Salmonella* in wash water and on mango surfaces. However, regular monitoring and replenishment of the disinfectant were critical to their sustained antimicrobial efficacy.

T1-06 Assessing Optimal Sanitization Procedures for a Postharvest Produce Brush Washer

AMANDA KINCHLA, Kelsi Harper and Catherine Gensler
University of Massachusetts-Amherst, Amherst, MA

Introduction: Produce brush washers are commonly used in small produce production, but are difficult to clean because of their mechanical design. This study investigated procedures to reduce microbial loads within this machine using approaches practical for small farms.

Purpose: This work aimed at identifying an improved method for cleaning and sanitizing a produce brush washer, which could be developed into a standard operating procedure that would be shared through extension programming to benefit growers.

Methods: Postharvest produce brush washing experiments were conducted by surface inoculating targeted zones on the equipment with nonpathogenic, streptomycin-resistant *Escherichia coli* MC4100 to compare different washing times and concentrations of sodium hypochlorite. Sponge Swabs (3M, Minneapolis, MN) were used for sample collection. Samples were serially diluted and plated on 3M®Aerobic Plate Count (APC) and *E. coli*/Coliform Petrifilm™. Experiments were conducted in triplicate and statistical analysis of variance (ANOVA) was performed.

Results: Results showed that up to five minutes of rinsing with a non-treated water in the brush washer yielded no statistical difference (*P=0.707*) compared to the initial inoculation. This indicated that the use of water alone is not sufficient to remove surface inoculated *E.coli* (control: 4.47 log CFU/96 cm^2; 300 second rinse: 3.56 log CFU/96 cm^2). Treatment with a five minute water rinse plus a treatment of 200 ppm sodium hypochlorite resulted a reported value of <25 CFU/96 cm^2 reduction. In addition, all chlorine concentration treatments were more effective at lowering *E. coli* levels than just a 30-second water rinse alone. *Escherichia coli* reductions after 200 ppm, 100 ppm, 50 ppm, and 25 ppm chlorine treatments were 4.31, 3.53, 2.68, and 1.78 log CFU/96 cm^2, respectively.

Significance: This work helped to identify optimal sanitizing conditions for a produce brush washer. This information will be used to develop a standard operating procedure for small scale production, which will improve food safety practices.

T1-07 Inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria innocua* Inoculated onto Grape Tomato, Spinach, and Cantaloupe with Aerosolized Hydrogen Peroxide

Yunbin Jiang[1], Kimberly Sokorai[2], Georgios Pyrgiotakis[3], Philip Demokritou[3], Xihong Li[1], Sudarsan Mukhopadhyay[4], Tony Jin[2] and **XUE-TONG FAN**[2]

[1]Key Laboratory of Food Nutrition and Safety (Tianjin University of Science and Technology), Ministry of Education, Tianjin, China, [2]U.S. Department of Agriculture-ARS, Eastern Regional Research Center, Wyndmoor, PA, [3]Center for Nanotechnology and Nanotoxicology, Harvard School of Public Health, Boston, MA, [4]U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA

Introduction: The traditional method of washing fresh produce with aqueous sanitizers has limited effectiveness, partially due to the failure of aqueous chemical sanitizers to reach pathogens that are often residing in the protected sites of plant tissue.

Purpose: The purpose of this study was to investigate the efficacy of aerosolized hydrogen peroxide in inactivating bacteria, while maintaining the quality of grape tomato, baby spinach leaves, and cantaloupe.

Methods: Stem scar and smooth surfaces of tomatoes, spinach leaves, and cantaloupe rinds, inoculated with *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria innocua*, were treated for 45 s followed by an additional 30 min dwell time with hydrogen peroxide (7.8%) aerosols activated by atmospheric cold plasma. Populations of surviving bacteria were recovered and enumerated.

Results: Two sizes of hydrogen peroxide droplets (mean diameters of 40 nm and 3.0 mm) were produced. The treatment reduced populations of the three bacteria on the smooth surface of tomatoes to nondetectable levels (detection limit 0.6 log CFU/fruit). However, on the stem scar area of tomatoes, the reductions of *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. innocua* were only 1.0, 1.4, and 1.2 logs, respectively. On the cantaloupe rind, the treatment reduced populations of *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. innocua* by 4.9, 1.3, and 3.0 logs CFU/piece, respectively. Under the same conditions, reductions achieved on spinach leaves were 1.5, 4.2, and 4.0 logs for *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. innocua*, respectively. The treatment, also, significantly (P<0.05) reduced populations of native aerobic plate count and yeasts and mold on tomato fruits and spinach leaves. Furthermore, firmness and color of the samples were not significantly (P>0.05) affected by the treatment.

Significance: Our results showed that the efficacy of aerosolized hydrogen peroxide depended on types of inoculated bacteria and produce. This technology could potentially be used to sanitize fresh fruits and vegetables.

T1-08 Control of Cross-contamination during Retail Handling of Cantaloupe

CHRISTOPHER RUPERT[1], Laura Strawn[2], Michelle D. Danyluk[3], Loretta Friedrich[3] and Benjamin Chapman[1]

[1]North Carolina State University, Raleigh, NC, [2]Virginia Tech - Eastern Shore AREC, Painter, VA, [3]University of Florida, Lake Alfred, FL

❖ ***Developing Scientist Competitor***

Introduction: Cantaloupe has been implicated in several outbreaks of foodborne diseases, including listeriosis and salmonellosis. The source of contamination for these outbreaks have historically been traced back to production or packing facilities. Little is known about the microflora found on cantaloupe contact surfaces in the food retail environment.

Purpose: The purpose of this study was to identify cantaloupe contact surfaces in retail environments for both whole and fresh-cut cantaloupe and determine the presence of microorganisms, including *Listeria*, on such surfaces.

Methods: A total of 141 environmental samples were collected using Dey/Engley sponge swabs from seven contact surfaces at four time points in five food retail locations during the fall of 2016. Swabs were homogenized with peptone water and plated on ECC for coliforms and *Eschericia coli*, TSA for total aerobic count, MOX for *Listeria* spp., and LMPM for *Listeria monocytogenes*.

Results: Approximately 32% (45 of 141) of swabs tested positive for *Listeria* spp.; no *L. monocytogenes* positive sample was recovered from any of the surfaces. Surfaces associated with whole cantaloupe contact had higher prevalence of *Listeria* spp. with 73.7% (28 of 38) testing positive. Surfaces associated with fresh-cut production had a comparatively lower prevalence of *Listeria* (17.9%; 17 of 95). One sample (0.71%) tested positive for *E. coli*. Surfaces that were regularly cleaned showed a decrease in coliform levels after sanitation. Whole cantaloupes were placed on top of foam to provide cushioning and prevent bruising. This foam had a high prevalence of *Listeria*, with 100% (19 of 19) swabs testing positive for *Listeria spp*.

Significance: No samples tested positive for *L. monocytogenes* and only one sample tested positive for *E. coli*. Additionally, cleaned surfaces showed a reduction in coliform counts, suggesting effective cleaning practices. Areas associated with whole cantaloupe use are more likely to be contaminated with *Listeria* spp. Foam, which was rarely cleaned, was found to be a harborage point for *Listeria* spp.

T1-09 Minimizing the Risk of Microbial Contamination in Berry Primary Production: From Theory to Implementation in Different Regions of the World

FRANÇOISE JULIEN-JAVAUX[1], John Donaghy[2], Jean-Jacques Lerouge[3], Liesbeth Jacxsens[4] and Sophie Zuber[1]

[1]Nestlé Research Center, Lausanne, Switzerland, [2]Nestec Ltd., Vevey, Switzerland, [3]Nestlé Professional, Vevey, Switzerland, [4]Ghent University, Ghent, Belgium

Introduction: In the recent past, fresh and frozen berries, grown in different regions of the world, have been implicated in foodborne outbreaks due to their contamination with pathogens, such as *Escherichia coli* O157, Hepatitis A, or Norovirus. Fresh and frozen berries are often used in food products without further processing prior to consumption. Therefore, it is crucial to minimize the risk of contamination early in the upstream supply chain: at the farm level from pre- to postharvest activities.

Purpose: The objective of this study were to (i) develop harmonized, science-based recommendations for farmers and to (ii) work together with farmers and processors to support implementation and assessment of these recommendations, and training for continuous improvement.

Methods: Current literature on Good Agricultural Practices (GAPs) and microbiological risk mitigation was reviewed, including peer-reviewed publications and guidelines and standards from governments, certification bodies, and NGOs. In parallel, farm visits were performed in different regions of the world, in order to convert these theoretical requirements into easily understood and implementable recommendations.

Results: A visual training booklet was developed, illustrating the best practices to follow in order to reduce the microbiological risk coming from seven routes of contamination. This booklet focuses on how to fulfill the requirements, which is often missing in generic guidelines. It provides new information that is not available elsewhere, such as a risk ranking of agricultural water according to water source and type of application. This booklet, which is available in several languages, is supplemented by posters and an assessment tool.

Significance: The usefulness and practical applicability of the booklet and its accompanying tools have been validated by berry farmers and processors in different global regions and acknowledged by National Food Safety Agencies. It provides very practical tools to ensure compliance with GAPs at the farm level and, thus, lowers the public health burden linked to fresh and frozen berries.

T1-10 The Use of Systems Thinking to Conceptualize Approaches for Co-managing Produce Production Environments for Food Safety, Conservation, and Profit

DANIEL WELLER and Martin Wiedmann
Cornell University, Ithaca, NY

❖ ***Developing Scientist Competitor***

Introduction: The Distinction-System-Relationship-Perspective (DSRP) framework for systems thinking enables stakeholders to share multiple analytical perspectives within a unified conceptual environment. Using DSRP, regulations, incentives, and stakeholder practices can be assessed from multiple disciplines, scales of operation, and time-horizons. The utility and flexibility of systems thinking can be best understood through a real-world application of DSRP. This presentation will introduce the concepts underpinning the DSRP approach, using examples from produce safety. Specifically, the talk will focus on how systems-thinking approaches have been used to understand trade-offs between food safety, conservation, and economics in produce production systems.

Purpose: DSRP provides a conceptual framework that is useful for comprehensively approaching wicked problems within food safety, such as comanaging produce production environments.

Methods: We examined the impact of various management decisions on food safety, conservation, and profit within produce production systems over the short- and long-term. We developed telescoping mental models that incorporated fine scale and landscape-level processes. These models incorporated multiple perspectives (e.g., growers, conservationists, food safety specialists), as well as findings from multiple fields (e.g., food safety, ecology, horticulture, economics).

Results: We were able to develop a holistic understanding of the ecological processes, as well as the economic and regulatory forces, that underpin the preharvest environment and drive grower decisions. This understanding allowed for the identification of research gaps (e.g., the impact of noncrop vegetation removal on pollination services), as well as potential comanagement strategies that maximize food safety while minimizing conservation and economic costs.

Significance: Systems thinking is a potential approach for enabling industry and government stakeholders to make informed decisions that consider both the short- and long-term effects of regulations, voluntary grower agreements, and on-farm management practices.

T1-11 Improvement of Bacterial Separation from Leafy Vegetables by Enzymatic Digestion

DANHUI WANG[1], Ziyuan Wang[2], Fei He[3] and Sam Nugen[1]
[1]Cornell University, Ithaca, NY, [2]University of Massachusetts Amherst, Amherst, NY, [3]Oregon State University, Corvallis, OR

❖ ***Developing Scientist Competitor***

Introduction: An accurate and effective method to separate bacteria from food matrices is essential to the rapid bacteria detection for food safety; however, there remain significant challenges for the improvement of bacterial separation. Bacteria, such as *Salmonella*, can strongly attach to the surface of food or internalize within the food matrices, making their separation extremely difficult. Traditional methods of separating bacteria from food, such as blending, shaking, and stomaching, may not be efficient at removing all the bacteria.

Purpose: The purpose of this study was to develop a rapid and effective method to improve the separation of *Salmonella* from spinach and lettuce by enzymatic digestion.

Methods: Enzymatic digestion by pectinase and cellulase was used to break down the structure of the leafy green vegetables. After digestion, immunomagnetic separation of *Salmonella* from the liquefied samples was conducted to achieve a pure sample without leaf debris. The optimal combination of pectinase and cellulase was investigated and the effectiveness of enzymatic digestion was compared with other methods (stomaching and manually shaking) on bacterial separation.

Results: The optimal combination of pectinase and cellulase provided an effective digestion for lettuce and spinach, resulting in the detachment and release of *Salmonella* from the leaves. The change in physical appearance and structure of the leaves demonstrated that the whole leaves were digested and became liquefied after enzymatic digestion. The recovery of the *Salmonella* from the spinach and lettuce after enzymatic digestion was significantly (P<0.05) higher (about twice) than the recovery when using traditional sample preparation methods (stomaching and manually shaking).

Significance: The results demonstrated the potential for use of enzymatic digestion prior to separation as a means to improve the efficiency of bacterial separation and increase the likelihood of pathogen detection in the final detection assay.

T1-12 Inactivation of *Salmonella*, Shiga-toxin Producing *Escherichia coli*, *Listeria monocytogenes,* Hepatitis A Virus, and Selected Surrogates on Frozen Blueberries by Candying

MATTEO CAMPAGNOLI[1], Xi Bai[2], Lise Michot[1], Thierry Putallaz[1], Sophie Butot[1], Frédérique Cantergiani[1], Mireille Moser[1] and Sophie Zuber[1]
[1]Nestlé Research Center, Lausanne, Switzerland, [2]Wageningen University, Wageningen, Netherlands

Introduction: Candied berry fruits are commonly used in the food industry as ingredients for ice creams, chocolates, and cereal based products. Most of these candied fruits undergo only mild thermal treatments and there are gaps to be filled in the industry to better understand the level of safety achieved towards vegetative pathogenic bacteria and viruses.

Purpose: This study was conducted to understand the level of inactivation achieved by candying (sugar infusion and consequent air drying) towards bacterial pathogens, HAV and selected surrogates. This will allow better assessment of risks and better management of the microbial safety throughout the berry supply chain.

Methods: Frozen blueberries were thawed and wet inoculated using fresh cultures of *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC), *Listeria monocytogenes,* and HAV, as well as the selected surrogates *Enterococcus faecium*, *Escherichia coli* P1, *Listeria innocua,* MS2, and the human Norovirus surrogate, MNV. Inoculated fruits were sugar infused to a final sugar concentration of 34 to 45% using different infusion temperatures (23, 35, 40, 45°C). After infusion samples were air dried, the surviving bacteria and viruses were enumerated and the level of inactivation was calculated.

Results: The sugaring process at 40°C was shown to achieve > seven and > six log CFU/g reduction for *Salmonella* and the other vegetative microorganisms, respectively. For MS2, 5.75±0.03 log PFU/g reduction were reported. For HAV and MNV, 1.46±0.22 and 3.40±0.17 log PFU/g reductions were reported, respectively, at 45°C. No reportable inactivation was observed at 23°C. The air drying of the sugared berries at 100°C for one hour delivered > five log CFU/g reduction on *Salmonella*, STEC, *E. faecium,* and *E. coli* P1. On HAV, MS2, and MNV, 2.13±0.23, 6.22±0.66, and 3.17±0.03 log PFU/g, respectively, were reported after air drying.

Significance: These data showed that mild processing like sugaring and air drying of blueberries can contribute an acceptable level of inactivation towards bacterial pathogens, HAV, and selected surrogates when specific processing parameters are applied.

T2-01 Enteroaggregative *Escherichia coli* is the Predominant Diarrheagenic *Escherichia coli* Pathotype among Irrigation Water and Food Sources in South Africa

MATTHEW AIJUKA[1], Araceli Santiago[2], Jorge Girón[3], James Nataro[4] and Elna Buys[1]

[1]University of Pretoria, Pretoria, South Africa, [2]University of Virginia, Charlottesville, VA, [3]University of Virginia, Charlottsville, VA, [4]University of Virginia School of Medicine, Charlottesville, VA

❖ ***Developing Scientist Competitor***

Introduction: Diarrheagenic *Escherichia coli* (DEC) has been implicated in foodborne outbreaks, worldwide, and have been associated with childhood stunting in the absence of diarrhea. Infection is common, but the routes of transmission have not been determined.

Purpose: Therefore, determining the most prevalent pathotypes, as well as associated food and environmental sources, may help provide better guidance to various stakeholders in ensuring food safety and public health.

Methods: PCR, partial gene sequencing, and cell adherence were used to characterize 205 *E. coli* strains previously isolated from milk (118), irrigation water (48), irrigated lettuce (29), and coleslaw (10) in South Africa, based on four diarrheagenic *E. coli* pathotypes; enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC).

Results: Using a combination of molecular and phenotypic assays, we identified only EAEC (37%, 17 out of 46) and EIEC (4.3%, 2 out of 46) pathogens in our samples. PCR and partial gene sequencing initially confirmed EAEC (2.4%, 5 out of 205) virulence gene determinants (*aatA* and *aaiC*) as the only pathotype. Phylogenetic analysis based on the identity of *aatA* and *aaiC* genes in strains from this study compared to those in Genbank showed close relatedness to foodborne and uropathogenic strains. Additionally, human strains clustered differently from environmental strains suggesting a potential role of virulence gene determinants in source tracking.

Significance: EAEC may be the leading cause of foodborne and waterborne enteric infection in South Africa. Using only molecular based methods targeting virulence gene determinants may underestimate numbers, especially among heterogeneous pathogens such as EAEC.

T2-02 Antibiotic Resistance Reservoir in Urban Agricultural Soils

ABDULLAH IBN MAFIZ, Liyanage Nirasha Perera, Shujie Xiao, Weilong Hao and Yifan Zhang

Wayne State University, Detroit, MI

❖ ***Developing Scientist Competitor***

Introduction: Soils are widely believed to be an important source of antibiotic resistance. Urban agriculture is host to multiple risk factors for antibiotic resistance and provides a unique model to investigate the extent of antibiotic resistance in the environment.

Purpose: This study was conducted to examine the extent of antibiotic resistance and metal resistance in urban agricultural soils.

Methods: Soil bacteria were isolated from 41 soil samples collected from an urban garden in Detroit. Antibiotic resistance profiles were determined using the Sensititre Antimicrobial Susceptibility System. Ten multidrug-resistant soil bacteria were selected for whole genome sequencing. Deep HISeq Illumina sequencing was performed on 21 soil samples. The load of antibiotic and metal resistance genes was expressed as number of open reading frames (ORF). Common antibiotic and metal resistance genes were identified via automated BLAST search.

Results: A total of 207 soil bacteria were isolated. Gram-negative bacteria (n=190) demonstrated resistance to ampicillin (94.2%), chloramphenicol (80.0%), and cefoxitin (79.5%). All gram-positive bacteria (n=17) were resistant to penicillin, gentamicin, and kanamycin. Whole genome sequencing showed the abundance of antibiotic and metal resistance genes. All soil samples (100%) carried resistance genes to quinolones, followed by β-lactam (95%), and tetracyclines (85.7%). The highest number of identified ORF was found in resistance genes to β-lactams (37.3%), macrolides (36.0%), and tetracyclines (18.4%). Antibiotic resistance genes, especially tetracycline resistance genes, in soil correlated positively with the MIC of soil bacteria. Arsenic, copper, and zinc resistance genes were present in all 21 samples. A strong positive correlation was found between total antibiotic resistance genes and total metal resistance genes (P=0.00). Zinc resistance genes were positively correlated with resistance genes to aminoglycosides (P=0.02), β-lactams (P=0.05), quinolones (P=0.03), and tetracyclines (P=0.00).

Significance: The data adds to our knowledge regarding extent of the environmental reservoir of antibiotic resistance and the possible co-selection of antibiotic resistance by metals in the environment.

T2-03 Effect of Antibiotic Withdrawal from Broiler Diets on Gut Microbiome and Foodborne Pathogen Prevalence

SANJAY KUMAR[1], Chongxiao Chen[1], Nagaraju Indugu[2], Gabriela Werlang[3], Manpreet Singh[1], Woo Kyun Kim[1] and Harshavardhan Thippareddi[1]

[1]University of Georgia, Athens, GA, [2]University of Pennsylvania, Kennett Square, PA, [3]Federal University of Rio Grande do Sul, Gonçalves, Brazil

Introduction: Due to development of antibiotic resistance by foodborne pathogens of poultry origin, *Salmonella* spp. and *Campylobacter* spp. are concerns to public health. There is a need to understand the effects of antimicrobial removal from feed on gut microbiota and the prevalence of foodborne pathogens.

Purpose: This study assessed the gut microbiome and concurrent prevalence of *Salmonella* spp. and *Campylobacter* spp. in the cecum and ileum of broilers.

Methods: A longitudinal study (hatch to market, day 42) was conducted to assess the gut microbiome and prevalence of *Salmonella* spp. and *Campylobacter* spp. in the cecum and ileum of chicks. Three diets, with and without bacitracin dimethyl salicyclate-50, were provided to the birds from day 0 to day 22 (starter diet), day 23 to day 35 (grower diet), and day 36 to day 42 (finisher diet). Cecal, ileum, litter, and feed samples were collected and sequenced using Illumina MiSeq.

Results: *Firmicutes* was the most abundant phylum in both cecum and ileum. The proportion of *Firmicutes* in the cecum decreased, gradually, with bird age (75.5% to 42.1% from day 0 to 42). *Proteobacteria* were abundant (18.8%) in the cecum at day 0, but varied in abundance with age. *Enterococcus* spp. was abundant in both the cecum and ileum on day 0, but decreased with age, from 54.0% to <1.0%. Day, diet, and treatment interaction (P≤0.0001) was significant for most genera, except members of the orders *Clostridiales* and *Lactobaccillales*. The prevalence of *Salmonella* spp. and *Campylobacter* spp. varied from 16.7 to 100.0% and 0 to 66.7%, respectively, in the cecum and ileum. Prevalence of *Salmonella* spp. and *Campylobacter* spp. in litter was 41.7% and 25.0%, respectively. Broiler age, diet, and treatment (control vs. antibiotic supplemented feed) affected the bacterial taxa in the gut.

Significance: The results of this study provided insight on the impact of antimicrobial supplementation in the feed on gut microbial composition and foodborn pathogen prevalence.

T2-04 Microbial Community Drivers of *Escherichia coli* O157 Colonization and Shedding in Early Lactation Dairy Cattle

CHLOE STENKAMP-STRAHM, Sheryl Magzamen, Craig McConnel, Zaid Abdo, Amanda VanDyke-Gonnerman, Joshua Schaeffer and Stephen Reynolds
Colorado State University, Fort Collins, CO

❖ ***Developing Scientist Competitor***

Introduction: Shiga-toxin producing *Escherichia coli* O157 (O157) infects upwards of 73,000 humans in the United States annually. Dairy cattle harbor and silently shed O157 in feces, playing a role in human pathogen exposure.

Purpose: Because cattle mount no O157 immune response and have uniform diets and environmental exposures, we hypothesized that the structure of the colonic microbial community influences a given animal's risk of shedding O157. The current study aimed to measure these microbial communities in early lactation dairy cattle, and model outcomes with O157 shedding metrics.

Methods: Cattle feces were collected from 40 O157 shedding and nonshedding dairy cattle on two farms over the course of five days. Samples were characterized for O157 using standard laboratory detection techniques. Based on detection, individual samples were classified for presence or absence of O157 and study cattle were classified by O157 shedding pattern (never, intermittent, multiday) and ever versus never shedding. 16s rRNA sequencing was used to measure microbial community composition. Alpha diversity metrics (richness, evenness and Shannon's index) were quantified and associated with shedding outcomes via regression modeling, adjusting for covariates previously seen to influence O157 shedding status (parity, days in milk, disease, farm and disease treatment). Differential abundances of bacterial taxa were identified between O157 positive and negative samples using zero-inflated Gaussian models.

Results: A total of 196 samples were analyzed for O157 presence. When outcomes were modeled with alpha diversity measures, multiday shedding animals were seen to have significantly lower richness than those that never shed O157. Zero-inflated modeling revealed 3 taxa more abundant in nonO157 samples: *Bacillus coagulans, Blautia producta,* and *Clostridium neonatale. Moryella indoligenes,* meanwhile, was seen to be more abundant in O157 positive samples.

Significance: These results indicate that both microbial diversity and some key taxa may influence O157 shedding of healthy dairy cattle. Understanding these microbial community changes may be used to guide on-farm strategies that mitigate O157 dissemination, ultimately protecting the human food chain.

T2-05 Characterization of Multidrug-resistant *Salmonella* Typhimurium and *Salmonella* Kentucky Strains Recovered from Chicken Carcasses Using Genotypic and Phenotypic Methods

SALINA PARVEEN[1], Rizwana Tasmin[1], Nur Hasan[2], Christopher Grim[3], Arquette Grant[4], Seon Choi[5], Mohammad Alam[3], Rebecca Bell[6], Christopher Cavanaugh[7], Kannan Balan[3] and Uma Badu[7]
[1]University of Maryland Eastern Shore, Princess Anne, MD, [2]CosmosID, Rockville, MD, [3]U.S. Food and Drug Administration, CFSAN, Laurel, MD, [4]Univerisity of Maryland Eastern Shore, Princess Anne, MD, [5]University of Maryland-College Park, College Park, MD, [6]U.S. Food and Drug Administration, College Park, MD, [7]U.S. Food and Drug Administration, Laurel, MD

Introduction: *Salmonella* Typhimurium (ST) is the leading cause of human non-typhoidal gastroenteritis in the United States. *Salmonella* Kentucky (SK) is one the most commonly recovered serovars from processed poultry carcasses. Considerable knowledge gaps still exist regarding the biology of multidrug resistant (MDR) ST and SK.

Purpose: The purpose of this study was to investigate the genotypic and phenotypic characteristics of ST and SK recovered from commercially processed chicken carcasses using whole genome sequencing, phenotype microarray, and intracellular killing assays.

Methods: One MDR ST (ST221_31B) and one MDR SK (SK222_32B) strain were sequenced using an Illumina MiSeq and compared with 28 previously sequenced *Salmonella* genomes. For phenotypic variation, 980 metabolic conditions were tested using the Biolog phenotypic microarray. Intracellular killing assays were conducted using chicken and murine macrophages.

Results: Phylogenetic analysis employing homologous alignment of a 1,185 non-duplicated protein-coding core genome demonstrated fully resolved, bifurcating patterns with varying levels of diversity and separated both ST and SK into distinct monophyletic serovar-specific clades. Single nucleotide polymorphism (SNP) analysis identified 1,911 SNPs within ST and 176 SNPs within SK strains. In addition to serovar-specific conserved coding sequences, the genomes of ST and SK harbor several genomic regions with significant genetic differences. These included phage and phage-like elements, carbon utilization or transport operons, fimbriae operons, putative membrane-associated protein-encoding genes, antibiotic resistance genes, siderophore operons, and numerous hypothetical protein-encoding genes. ST was capable of utilizing certain carbon compounds more efficiently than SK. ST survived for 48 h in macrophages, while SK was mostly eliminated. A three-fold growth of ST was observed in chicken granulosa cells, while SK was unable to replicate in these cells.

Significance: These results demonstrated that ST can survive host defenses better and is more invasive than SK and provided some insights into the genomic determinants responsible.

T2-06 Contribution of Alternative Sigma Factors on *Listeria monocytogenes* Survival in Synthetic Bile

ATSADANG BOONMEE[1], Soraya Chaturongakul[1] and Haley Oliver[2]
[1]Mahidol University, Bangkok, Thailand, [2]Purdue University, West Lafayette, IN

Introduction: *Listeria monocytogenes* can persist and grow under extreme conditions, such as low pH in the stomach and bile in the intestinal fluid. The ability to survive exposure to bile is important for subsequent colonization and infection. *Listeria monocytogenes* regulates gene expression patterns in response to stressors, during gastrointestinal passage, through use of alternative sigma factors (σ).

Purpose: The purpose of this study is to determine the role of alternative sigma factor(s) (σ^B, σ^C, σ^H, σ^L) in *L. monocytogenes* stress response and survival in synthetic bile juice. We hypothesized that coregulation of sigma factors allowed *L. monocytogenes* to survive in bile stress and, subsequently, establish infection.

Methods: *Listeria monocytogenes* parent strain 10403S and isogenic single, double, triple, and quadruple deletion mutants were grown in brain heart infusion (BHI) broth at 37°C overnight. Cultures were transferred to fresh BHI broth and grown to midlog phase (OD_{600}= 0.4), then exposed to synthetic bile

juice (pH 8.2) for 10 and 20 min. Survival was determined by serial dilution and plating on BHI agar. Experiments were conducted in triplicates. Differences in survival abilities were determined using the t-test.

Results: $\Delta\sigma^B$ and $\Delta\sigma^C$ mutants showed significant decreases in survival in comparison to wildtype after 10 min exposure (*P*=0.010 and 0.036, respectively). $\Delta\sigma^H$ and $\Delta\sigma^L$ mutants survived significantly better than wildtype after 10 min (*P*=0.005 and 0.002, respectively). Moreover, among the alternative sigma factors, σ^L showed the greatest significant effect on *L. monocytogenes* survival abilities in synthetic bile.

Significance: The data suggested that σ^B and σ^C have positive effects, while σ^H and σ^L have negative effects on *L. monocytogenes* survival in bile. Expression of genes under σ^B and σ^C regulation could potentially promote *L. monocytogenes* adaptation during gastrointestinal passage and, therefore, may be important control strategy targets.

T2-07 Phenotypic and Pan-genomic Characterization of *Salmonella enterica* serovar Uganda, an Uncommon Foodborne Pathogen

DANIEL HURLEY[1], Maria Hoffmann[2], Ellen Wall[1], Eric Brown[2], Marc Allard[2], Salim Mattar[3] and Séamus Fanning[1]

[1]University College Dublin, Dublin, Ireland, [2]U.S. Food and Drug Administration, College Park, MD, [3]Universidad de Córdoba, Córdoba, Colombia

❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* Uganda is an uncommon serovar rarely isolated from humans. It has been implicated in only three foodborne outbreaks reported in the United States between 1993 and 2012. Little is known about its genetic diversity or pathogenicity of this bacterium.

Purpose: The purpose of this study was to phenotypically characterize 14 isolates and study the pan-genome of *Salmonella* Uganda.

Methods: Intracellular survival of select isolates in human THP-1 macrophages was comparatively assessed using *Salmonella* Typhimurium. Macrophage proinflammatory cytokine and chemokine markers were quantified postinfection. Whole genome sequencing was performed using the Illumina MiSeq platform. A high-quality reference genome for *S*almonella Uganda CFSAN006159 was generated on the Pacific Biosciences RS II platform.

Results: In THP-1 macrophages, *Salmonella* Uganda CFSAN006159 recorded a <two-log reduction between two and 168 hours post infection, whereas *Salmonella* Uganda CFSAN006173 recorded a < one-log reduction over the same time course. Both *Salmonella* Uganda isolates persisted for seven days within human macrophages, unlike the *Salmonella* Typhimurium references which were unrecoverable. Infection with *Salmonella* Uganda stimulated increased cytokine (CXCL8, IL1B and TNF) and chemokine (CCL2, CCL3 and CCL22) release compared to the reference strain.

SPI-13, containing the *lgl-ripABC* operon in addition to three uncharacterized genes, has currently been reported to be highly upregulated only within macrophages. *Salmonella* Uganda CFSAN006159, which has been shown in this study to survive readily in THP-1 macrophages in addition to eliciting a large proinflammatory response, uniquely harbours two complete chromosomal copies of SPI-13, located approximately 460-kbp apart. Bioinformatic analyses suggests that these loci appear to have been acquired from distinct genetic lineages. This finding may help explain the extreme pathogenicity of this isolate during infection as a result of gene dosage effect.

Significance: Characterizing the phenotypic virulence and genomic diversity of this serovar further extended our understanding of its ability to illicit a host-cell response during infection.

T2-08 Complete Genome Sequence of the Thermotolerant Foodborne Pathogen *Salmonella enterica* Serovar Senftenberg ATCC 43845 and Phylogenetic Analysis of Loci Encoding Thermotolerance

SCOTT NGUYEN, James Bono, Timothy Smith, Gregory Harhay and Dayna Harhay

USDA ARS U.S. Meat Animal Research Center, Clay Center, NE

Introduction: Previous studies in *Cronobacter sakazaki*i, *Klebsiella* spp., and *Escherichia coli* have identified a genomic island that confers thermotolerance to its hosts. This island has recently been identified in *Salmonella enterica* serovar Senfentenberg ATCC 43845, a historically important, heat resistant isolate. Comprehensive analyses of the genetic mechanisms contributing to the thermotolerance of this strain have been impaired, as the complete genome sequence has not, previously, been available.

Purpose: This study was undertaken to characterize the complete genome sequence of *Salmonella* Senftenberg ATCC 43845, in order to facilitate our understanding of the genetic determinants contributing to the thermotolerance of this strain, and to conduct a phylogenetic analysis of thermotolerance loci, in order to examine their distribution among the Proteobacteria.

Methods: Long-read, single molecule, real time sequencing technology was used to obtain the complete chromosome and finished quality assembly of *Salmonella* Senftenberg ATCC 43845. Thermal tolerance loci identified in *Salmonella* Senftenberg were used to identify related islands in the GenBank nr (nonredundant) database using BLASTn. Loci were aligned using MAFFT and the resulting aligned sequences were analyzed by jModelTest 2 to determine parameters for MrBayes 3.2.6 analysis, which was used to calculate a Bayesian inference tree of the loci.

Results: Complete genome sequence analysis revealed that *Salmonella* Senftenberg ATCC 43845 contains not one, but two copies of a thermotolerance island on a 341.3 kb IncHI2 plasmid. Phylogenetic analysis of these loci revealed three clades with a sparse, yet broad distribution in the *Proteobacteria* and sheds light on the evolution and distribution of loci conferring resistance to environmental stressors such as heat and desiccation.

Significance: This is the first report that *Salmonella* Senftenberg ATCC 43845 contains not one, but two copies of a thermotolerance island on a 341.3 kb IncHI2 plasmid. The data presented expand our understanding of the distribution and evolution of genetic mechanisms of heat resistance in foodborne bacterial contaminants.

T2-09 Surveillance of the *Listeria monocytogenes* Profile of an Irish Food Processing Facility Over Five Years Using Whole-genome Sequencing

LAURA LUQUE-SASTRE[1], Craig T. Parker[2], Steven Huynh[2] and Séamus Fanning[1]

[1]University College Dublin, Dublin, Ireland, [2]Western Regional Research Center, Produce Safety and Microbiology Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Albany, CA

❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* is the third leading cause of death among bacterial foodborne pathogens in the United States. It is commonly found in food and processing facilities, where it can persist. Its ability to survive under a wide range of environmental conditions enhances the potential of cross-contamination to final products, leading to possible outbreak events.

Purpose: Here, whole genome sequencing (WGS) was applied as a surveillance tool to track and characterize *L. monocytogenes* in a food processing company.

Methods: One hundred *L. monocytogenes* isolates from environmental and food samples were studied. These were sequenced using a MiSeq platform, de novo assembled using SPAdes, and the genomes were annotated using Prokka. The genomes were used to (i) perform core genome multilocus se-

quence typing (cgMLST) and single nucleotide polymorphism analysis to investigate persistence, (ii) detect the presence of antimicrobial resistance-encoding genes, and (iii) analyze the occurrence of mutations in the major virulence factors.

Results: *Listeria monocytogenes* isolates were classified in 18 different clonal complexes (CC), the most prevalent being CC101 (21%), CC9 (17%) and CC121 (12%). Ten cgMLST types were found to be putatively persistent, as they were identified at least three times within a 12-month period. Benzalkonium chloride tolerance genes were found in 59% of the isolates, the most prevalent gene was *emrC* followed by *bcrABC*, *qacH*-Tn*6188* and *qacC.* The *L. monocytogenes* major virulence factor, *inlA* was truncated in 31% of the isolates and one environmental isolate harboured all major virulence factors, including the PTS system, which has been shown to confer hypervirulence.

Significance: This study provided a further understanding of the genetic diversity and mutations among isolates within the product and environment of one food processing company. Furthermore, WGS was an excellent tool to assess pathogenic genotypes of *L. monocytogenes*. This study highlighted the potential for WCS application as a prospective means of *L. monocytogenes* surveillance and could be used for other pathogens relevant to Public Health.

T2-10 Virulence Genes and Multi-drug Efflux Pumps are Differentially Expressed in *Salmonella* Heidelberg Exposed to Heat Shock

ANDREA RAY and Haley Oliver
Purdue University, West Lafayette, IN

❖ ***Developing Scientist Competitor***

Introduction: Multidrug resistant *Salmonella* Heidelberg in poultry was responsible for a significant outbreak in 2013 to 2014. We found that a food-associated isolate from this outbreak, PUL R1-0006, had significantly higher heat resistance compared to *Salmonella* Heidelberg, reference strain SL476.

Purpose: The purpose of this study was to define and compare transcriptomes of PUL R1-0006 and SL476, exposed to heat shock mimicking scald temperatures, to investigate which genes may be contributing to survival and enhanced virulence of *Salmonella* Heidelberg.

Methods: RNA from each strain was extracted, in triplicate, from control (37°C) and heat shocked (7.5 min at 56°C) stationary-phase cultures using standard Trizol and rRNA-depletion methods. RNA quality and DNA and rRNA depletion were assessed. Libraries were constructed using the Illumina Scriptseq v2 library kit and sequenced on an Illumina HiSeq. Reads were trimmed and aligned; counts were obtained using HTSeq (v.0.6.1). Differential expression was determined using DESeq2 (v.1.12.4) in R (v3.3.1). Genes with $\log_2$-fold change >1 and adj. $P<0.01$ were considered significantly, differentially expressed (DE).

Results: In PUL R1-006, known heat shock-associated genes *dnaJ* and *rpoH* were upregulated, while *groS* was downregulated. Interestingly, a different subset of heat shock-associated genes *rpoH* and *hscAB* were upregulated and *ibpA* was downregulated in SL476. Twenty-four and 37 virulence-associates genes were DE in PUL R1-0006 and SL476, respectively, including genes in *Salmonella* pathogenicity islands (SPI-1 and SPI-2). Sixteen antibiotic resistance genes or efflux pumps were DE in PUL R1-0006; 15 were identified in SL476.

Significance: This is the first study to capture the transcriptome of *Salmonella* Heidelberg. Preliminary analyses suggested that exposure of *Salmonella* Heidelberg to heat shock conditions relevant to poultry processing may result in increased virulence and tolerance to antibiotics, which could partially explain the severity of disease associated with this serovar. Further studies are necessary to explore this relationship.

T2-11 Differentiation of Live and Dead *Escherichia coli* O157:H7 Using a PCR-based Method Combined with DNA Photo Labeling

AMY JONES, K.C. Jeong, Keith Schneider and Soohyoun Ahn
University of Florida, Gainesville, FL

❖ ***Developing Scientist Competitor***

Introduction: The CDC estimates Shiga toxin-producing *Escherichia coli* (STEC) causes over 265,000 infections and 30 deaths each year in the United States. *Escherichia coli* O157:H7 is the most common STEC serotype, being responsible for approximately 36% of those illnesses. While only viable *E. coli* O157:H7 cells can cause illness, presence of dead cells can result in false-positive results in many detection methods. DNA photo-labeling only neutralizes DNA from dead cells and, thus can selectively amplify DNA from live cells.

Purpose: The purpose of this study was to develop and optimize a rapid, PCR-based detection method combined with DNA photo-labeling able to differentiate live and dead *E. coli* O157:H7.

Methods: The procedure was optimized for full neutralization of dead cells, while maintaining amplification of live cells. Both live and dead *E. coli* O157:H7 (a farm isolate) culture samples were treated with or without DNA photo-labeling dye ethidium monoazide (EMA). Samples were then exposed to LED light and analyzed using multiplex PCR (mPCR) and quantitative PCR (qPCR).

Results: Under the optimized DNA photo-labeling condition of five-minute incubation with 25 µM EMA followed by five-minute of high-intensity LED light exposure, DNA from dead cells was neutralized and PCR amplification occurred only with DNA from live cells. Live cells were successfully differentiated from dead cells with a detection limit of 10^5 CFU/ml in mPCR. In qPCR where 10^6 to 10^8CFU/ml were tested, it was noted that the lower cell numbers gave better differentiation between dead and live cells.

Significance: This data suggested that DNA photo-labeling combined with PCR-based detection methods can differentiate live and dead *E. coli* O157:H7.

T2-12 Molecular Epidemiology of an Emerging Strain of *Salmonella enterica* serotype Infantis in the United States

JESSICA CHEN[1], Allison Brown[2], Lee Katz[2], Davina Campbell[2], Heather Tate[3] and Jason Folster[2]
[1]IHRC, Inc., Decatur, GA, [2]Centers for Disease Control and Prevention, Atlanta, GA, [3]U.S. Food and Drug Administration, Laurel, MD

Introduction: In July, 2015, FDA notified CDC of a CTX-M-65 producing isolate of *Salmonella* Infantis with a rare pulsed field gel electrophoresis pattern, JFXX01.0787, recovered from retail chicken meat. CTX-M confers resistance to 3rd generation cephalosporins including ceftriaxone, an important antibiotic used to treat invasive salmonellosis.

Purpose: The purpose of this study was to characterize a resistant strain of *Salmonella* Infantis by whole genome sequencing (WGS) and to better understand associated risk factors.

Methods: CDC performed WGS on 34 human clinical isolates of *Salmonella* Infantis pattern JFXX01.0787 and examined associated epidemiological data. Resistance determinants were identified using ResFinder. A high quality SNP (hqSNP) phylogeny was generated using Lyve-SET and a molecular clock analysis was implemented in BEAST.

Results: Epidemiological data showed an association with travel to South America in some clinical cases. WGS revealed that 29 of the 34 isolates possessed $bla_{CTX\text{-}M\text{-}65}$. The hqSNP analysis revealed that the majority of isolates formed a well-supported clade that also included the retail chicken isolate. Isolates in this clade differed by no more than 48 hqSNPs and travel and nontravel isolates did not cluster separately. These *Salmonella* Infantis isolates diverged from a common ancestor around February, 2006 (95% HPD Interval: 8/1/2004 to 2/23/2011), prior to the first isolation of pattern JFXX01.0787 in 2012. Examination of the resulting maximum-clade credibility tree, with tree tips labeled with travel status, revealed that isolates from patients with a history of travel were recovered consistently over the study period (2012 to 2015), whereas domestic cases with no history of travel first emerged in 2014.

Significance: Epidemiological data indicated that travel is a risk factor for exposure to this strain. Further, the close genetic relationship of a domestic chicken isolate with several clinical isolates in this study suggested that chicken may play a role in transmission of this strain.

T3-01 Changes in Food Handling Following a Food Safety Intervention among High School Students (Ontario, Canada)

KENNETH DIPLOCK[1], Andria Jones-Bitton[2], Scott Leatherdale[1], Steven Rebellato[3], Joel Dubin[1], David Hammond[1] and Shannon Majowicz[4]

[1]School of Public Health and Health Systems, University of Waterloo, Waterloo, ON, Canada, [2]University of Guelph, Guelph, ON, Canada, [3]McMaster University, Hamilton, ON, Canada, [4]University of Waterloo, Waterloo, ON, Canada

Introduction: High school students are a unique audience for interventions aimed at improving safe food handling and preparation. This demographic is increasingly responsible for handling and preparing food, such that teaching of safe food handling at this age may help instill lifetime safe food handling habits.

Purpose: The objective of this research was to observe how food safety practices of high school students changed following delivery of a provincial food handler training program, in Ontario, Canada.

Methods: We conducted an in-depth survey and observed a recipe preparation in eight Grade 10 and 12 food and nutrition classes in four Ontario high schools (n=119 students), assessing food safety knowledge, attitudes, and self-reported and observed food handling practices. The survey and observation were conducted at baseline (February, 2015; T_1), and repeated circa 2 weeks (T_2) and 12 weeks postintervention (T_3). The intervention was a truncated version of Ontario's provincial food handler training program, modified to omit commercial food premise specific content.

Results: Food handling practices were poor at baseline, with students averaging 50% correct behaviors (16 of 32). At T_2, behaviors improved significantly (by 4.4 points out of 32, P<0.0001), while behavior scores did not change significantly, between T_2 and T_3. At baseline only 5.5% (6 out of 108) of students used a probe thermometer to check doneness of their chicken. Student characteristics were not significant predictors of total food handling behaviors, while working or volunteering in a food service premise was found to be a significant predictor (P=0.025) of the use of a food thermometer.

Significance: Students' observed safe food handling was significantly better following delivery of a provincial food handler training program, suggesting that delivery of such material in food and nutrition classes may be an important strategy to ensuring food safety skills as students transition to adulthood.

T3-02 Food Safety Attitudes and Self-reported Behaviours of Undergraduate Students from a Canadian University

SARAH COURTNEY, Ashok Chaurasia, Kitty Corbett and Shannon Majowicz

University of Waterloo, Waterloo, ON, Canada

❖ ***Developing Scientist Competitor***

Introduction: In Canada, approximately four million cases of foodborne disease occur each year and an increase in foodborne disease incidence and prevalence in young adults has been observed. Studies of young adults from other countries show that they tend to have positive food safety attitudes and poor self-reported behaviours, but similar information is not available for Canada.

Purpose: The objective of this research was to explore food safety attitudes and self-reported behaviours of young adults in Canada, by conducting a pilot survey of undergraduate students at the University of Waterloo, Ontario, Canada.

Methods: In February, 2015, we administered an electronic, cross-sectional survey to a random sample of undergraduate students (n=485) at the University of Waterloo and assessed food safety attitudes and self-reported behaviours using questions from existing questionnaires. Data were analysed descriptively and using multiple linear regression.

Results: In general, students had positive food safety attitudes, with over 80% confident or very confident that they can cook safe and healthy meals for themselves and their families. Cooking experience was one of the few demographic variables that was significantly associated with food safety attitudes. Students' self-reported food safety behaviours varied. For example, the majority reported always (57%) or often (34%) washing hands with warm running water and soap after working with raw meat or chicken; however, the majority also reported never (32%) or rarely (50%) using a thermometer to check if leftovers have been reheated enough.

Significance: Undergraduate students appear confident in their ability to cook safe and healthy meals, but their varied self-reported behaviours indicate a potential need for enhanced food safety skills. The potential role of universities in providing food safety education or enhancing safe food handling in the Canadian context warrants further investigating.

T3-03 Educating Tailgaters on Best Food Safety Practices at College Football Tailgates

MARY YAVELAK[1], John Luchansky[2], Anna Porto-Fett[2], Jill Hochstein[3] and Benjamin Chapman[1]

[1]North Carolina State University, Raleigh, NC, [2]U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, [3]University of Nebraska-Lincoln, Lincoln, NE

❖ ***Developing Scientist Competitor***

Introduction: Preliminary research shows the majority of participants at collegiate football tailgates in the United States do not report using a food thermometer while preparing meat. Food preparation at temporary events like tailgates, community gatherings, and festivals requires implementation of safe food handling practices as such atypical settings can increase the risk of foodborne illness.

Purpose: Project objectives focused on educating consumers about safe food handling practices using hands-on demonstrations at a NC State University tailgate in November, 2016.

Methods: Following a semistructured script, trained individuals educated participants on proper food thermometer use; temperature of meat versus meat color; cleaning and sanitizing of utensils and work surfaces; and personal hygiene. Each station accommodated small groups of four to eight people, which allowed participants to discuss the topic and ask questions. Participants were also given a food thermometer and additional food safety information.

Results: Efficacy of demonstrations was assessed using surveys on tailgaters' thermometer use, including technique, frequency, and food products before and after the demonstration. In total (n=257), 57.6% of participants reported using a food thermometer before the demonstration, and 42.4% denied

use of a food thermometer. Only 15.4% of participants who reported using a food thermometer (*n*=148) reported use all the time while cooking meat. Of those who reported using food thermometers (*n*=148), most were used when cooking pork (74.3%), beef (68.9%), and chicken (60.8%), when participants could choose all that apply. Follow-up showed changes in thermometer use, knowledge, and self-reported practices.

Significance: As many nonprofessional food preparers cook food at temporary events, education of safe food handling practices is essential to reduce the risk of foodborne illness. Results from long-term data collection will provide insight on the effectiveness of the education demonstration so it can be optimized and tested as a large-scale food safety intervention.

T3-04 Safe Food Handling Behaviors of Student Volunteers in an On-campus Food Reclamation Program

HARRY SCHONBERGER[1], Renee Boyer[1] and Melissa Chase[2]

[1]Virginia Tech, Blacksburg, VA, [2]Virginia Tech/Virginia Cooperative Extension, Blacksburg, VA

❖ ***Developing Scientist Competitor***

Introduction: The Campus Kitchen is a national program supported at colleges and universities in the United States as a way for students to combat hunger by reclaiming surplus food from the university and distributing it in their community. At Virginia Tech, Campus Kitchen has engaged over 300 students to divert over 23,340 pounds of surplus food from waste streams in on-campus dining facilities since launching in September, 2015. Student volunteers either deliver food directly to two community clients or repurpose it into side dishes and desserts for them. These students have completed different levels of food safety training that supports their work, ranging from the ServeSafe® Manager certification through an academic course, to training through Dining Services, to not receiving any training prior to volunteering.

Purpose: The purpose of this study was to assess the effectiveness of current food safety trainings on the food handling behaviors of student volunteers and to determine if there are additional training needs for this audience.

Methods: Observations were conducted with students volunteering at each level of the program (food diversion, delivery, and cooking shifts); their self-reported safe food handling practices were recorded via online survey to determine consistency with their observed behaviors.

Results: Volunteers, regardless of their level of training, were observed engaging in unsafe food handling behaviors. Those who participated in formalized food safety training were more aware of their behaviors. Students believed they would benefit from additional training, which should be developed for their continuing education.

Significance: With growing interest in food reclamation and access, practicing safe food handling is a key need for these programs. This study highlights gaps in food safety training for this specific food handler audience.

T3-05 An Evaluation of Food Safety Culture and a Training Intervention: Getting the Most Out of Your Training Program

KRISTEN SANIGA and Clint Stevenson

North Carolina State University, Raleigh, NC

❖ ***Developing Scientist Competitor***

Introduction: Internal food safety training is performed by many food companies to satisfy federal and/or private industry-driven requirements, yet little research has been done to study how to increase the effectiveness of internal food safety training to increase knowledge gains and improve attitudes and behaviors of employees.

Purpose: The purpose of this study was to build and validate an instrument to measure food safety attitudes and knowledge of employees before and after a food safety training intervention.

Methods: Twenty operators at a dairy processing facility were surveyed immediately before and after receiving allergen control training. Food safety culture evaluation questions were constructed to measure organizational characteristics; employee attitudes, knowledge, perceptions, and risk awareness; and group characteristics. Training evaluation questions were constructed using the Integrated Behavior Model. The analysis tool also involved collecting supervisor audit and regulatory audit data to measure food safety performance.

Results: Of the twenty responses collected, sixteen were usable for analysis. Subject matter experts and members of industry were referenced to ensure content validity. The training intervention had no effect on participants' attitudes, perceived norm, or personal agency. Pretraining responses were compared to pre- and posttraining knowledge scores and a correlation (53 to 72%) was found between posttraining knowledge scores and scores for injunctive-norm. A linear regression showed a positive correlation between injunctive norm and posttraining knowledge scores ($P<0.05$).

Significance: This data hints that training may be more effectively implemented if employees feel that certain food safety behaviors are expected by their peers, managers, and others. The data from this study warrants more research to study the relationship between training and food safety attitudes and perceptions.

T3-06 "Wash Your Produce": Determination of the Efficacy of a Piloted Food Safety Intervention at the Farmers' Market

SHAUNA HENLEY[1], David Martin[1], Jack Fu[2], Deanna Baldwin[3] and Shelby Watson-Hampton[3]

[1]University of Maryland Extension, Baltimore County, Cockeysville, MD, [2]Johns Hopkins Bloomberg School of Public Health, Department of Biostatistics, Baltimore, MD, [3]Maryland Department of Agriculture, Annapolis, MD

Introduction: Produce is a leading commodity identified in foodborne outbreaks nationwide. Previous formative research consisting of a semistructured interview with growers (*n*=6) and a consumer survey (*n*=230), determined that consumers demonstrate inconsistent knowledge and behavior of the best practice to wash fresh produce with clean running water. This conclusion warranted the creation of targeted materials.

Purpose: The aim of this study was to generate a reproducible, interdisciplinary approach to address safe produce-handling communication at farmers' markets during the point of purchase, when vendors may have limited time to discuss best handling practices with customers.

Methods: A local artist worked closely with researchers to create eye-catching signage that would be appropriate at farmers' markets, using health literacy principles. Two control farmers' markets (no educational signage) and two experimental farmers' markets (with the educational signage) were identified in Baltimore, Maryland. Signage was on display for four weeks, followed by a brief survey administered at each participating farmers' market.

Results: Market vendors voluntarily displayed a 12" by 18" color sign emphasizing produce washing at their location. Respondents at both the control (*n*=160) and experimental (*n*=166) sites did not believe that organic produce could have germs/bacteria that could make people sick. Respondents at the experimental sites were significantly ($P<0.05$) more likely to eat raw, unwashed produce at the farmers' markets, compared to respondents at the control sites. Younger respondents, between 25 to 44 years of age, were significantly more likely to engage in unsafe practices compared to older respondents.

Significance: This study determined that adherence to washing produce was generally high among both the control and experimental sites. However, farmers' markets seem to be an appropriate location for consumer food safety education to occur.

T3-07 An Assessment of Produce Growers' Sanitizer Practices and Knowledge about Antimicrobial Resistance

VAISHALI DHARMARHA[1], Monica Ponder[1], Renee Boyer[1], Laura Strawn[2], Tiffany Drape[1], Joell Eifert[1], Amber Vallotton[1] and Amy Pruden[1]
[1]Virginia Tech, Blacksburg, VA, [2]Virginia Tech - Eastern Shore AREC, Painter, VA

❖ ***Developing Scientist Competitor***

Introduction: Bacteria isolated from triple-washed, bagged, ready-to-eat leafy vegetables have been shown to possess antimicrobial resistance. Sanitizers used in produce washing may select for survival of resistant strains. Use of sanitizers in produce washing is a common practice. There is little known about how much produce growers understand about antimicrobial resistance and how their practices could influence antimicrobial resistance. It is critical to understand growers' sanitizer use practices and their knowledge of how these practices impact antimicrobial resistance in order to develop effective educational materials and fill knowledge gaps.

Purpose: The purpose of this study was to understand how produce growers utilize sanitizers in their operations, and to assess their general knowledge of antimicrobial resistance.

Methods: A survey was delivered to produce growers at grower meetings conducted by Virginia Cooperative Extension across Virginia. The tool inquired about their sanitizer use, as well as their understanding of antimicrobial resistance. Growers were, also, asked about their preferred delivery method for educational materials.

Results: Growers surveyed (*n*=61) generally understand that antimicrobial resistance is a global public health concern (61%). However, their understanding of how using sanitizers to wash fresh produce impacts antimicrobial resistance was less clear. Chlorine was the most common sanitizer used (65%), and the most common application method used to deliver sanitizer was through a spray bar (47.8%). Ninety-one percent of growers received sanitizer information from a chemical representative or local extension agent and 78% indicated extension agents and growers meetings as their preferred source for education.

Significance: It is clear that there are some knowledge gaps with this population related to how their practices impact antimicrobial resistance. Results demonstrate the importance of Extension as a delivery system for fresh produce growers. Extension agents could be used to deliver information on how using sanitizers can reduce antimicrobial resistance in the food supply.

T3-08 Mug Cakes Baked in Microwave Ovens: The Influence of Baking Time and Internal Temperature on Risk of Foodborne Illness

SARAH COPE, Natalie Seymour, Mary Yavelak and Benjamin Chapman
North Carolina State University, Raleigh, NC

❖ ***Developing Scientist Competitor***

Introduction: A recent *Escherichia coli* outbreak related to raw flour led to 63 illnesses in 24 states. Concurrently, there is a growing popularity of microwave baking on social sharing media sites, such as Pinterest. Following inaccurate baking information on these posts could result in an undercooked product resulting in increased illness risk.

Purpose: This study was conducted to determine whether the risk of foodborne illness resulting from improperly microwaved cakes may be increased due to the lack of time and temperature data on such recipes, limitations of microwave ovens, and the paucity of food safety messaging on the blog sites linked to Pinterest.

Methods: A protocol was developed to compile popular mug cake recipes from Pinterest. After analyzing recipes for food safety messaging and overall popularity (based on predetermined criteria), the most popular mug cake recipe was tested in triplicate. Temperature was measured on the surface, bottom, and center every 15 s of cooking and after the full baking time was completed.

Results: A total of 316 recipe pins were compiled from Pinterest; 31 provided inaccurate food safety information. A pilot study resulted in an average temperature of 188°F in the center, 163°F at the bottom, and 128°F on the surface, at the minimum baking time recommendation of 60 s. At the maximum baking time recommendation of 90 s, the cake reached an average temperature of 208°F in the center, 197°F at the bottom, and 168°F on the surface.

Significance: This exploratory project showed misinformation about the safety of microwave baking on social sharing media. Results suggest that a microwave mug cake can be baked safely when the maximum recommended baking time is followed, but further study should be done to compare different microwaves and recipes.

T3-09 Needs Assessment of Educational Intervention for Artisan Cheesemakers in the United States

MADHUMEETA DUTTA and Clint Stevenson
North Carolina State University, Raleigh, NC

❖ ***Developing Scientist Competitor***

Introduction: Over the past four decades there has been tremendous growth (~2,000%) in the number of artisan cheese facilities in the United States. Increased consumption of artisan cheese and the new requirements of FSMA rules have necessitated the need to look into the food safety hazards associated with artisan cheese. In August, 2015, members of an artisan dairy forum achieved consensus that, due to time and money constraints of the cheese makers, an online self-paced training intervention would be a good medium to educate artisans on basics of food safety.

Purpose: This study was conducted to determine the food safety training needs of small artisan cheesemakers in the US and to develop an intervention. This was based on the front end analysis for the instructional design to help them produce safe and wholesome cheese for their consumers.

Methods: A survey was sent to 85 individuals pulled from the national artisan dairy forum with a response rate of 29.4% (*n*=25). Qualitative data were, also, collected through phone, email, and face-to-face interviews.

Results: There are competency gaps in various food safety practices related to artisan cheese facility. According to the respondents of the survey the likelihood of having a food safety plan for large and medium sized operations are high (*n*=14 and *n*=10, respectively); whereas, small and very small operations are very unlikely (*n*=12). The survey also suggested that the artisan cheese makers have access to the internet (*n*=21) and use computer (*n*=21) and smartphone (*n*=20) for day-to-day use. Of the respondents, 11.76% (*n*=2) reported that no food safety training opportunities are offered to cheese makers in their regions.

Significance: The results indicated that the artisan community may lack basic food safety knowledge such as GMPs, pertinent laws and regulation, and they need basic food safety training.

T3-10 Thermometer Usage Behaviors for Thanksgiving Turkeys: Analysis of Data Collected by Citizen Scientists

MINH DUONG[1], John Luchansky[2], Anna Porto-Fett[2], Caitlin Warren[3] and Benjamin Chapman[1]
[1]North Carolina State University, Raleigh, NC, [2]U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, [3]Souderton High School, Souderton, PA

❖ ***Developing Scientist Competitor***

Introduction: Self-reported behavioral data is unreliable because of exaggeration and social desirability bias. The incorporation of pictures provides a different medium for understanding data by presenting tangible evidence of the data collector's perspective to the researcher. Citizen science is a unique intervention method where nonscientists gather and interpret data in collaboration with professional scientists.

Purpose: The purpose of this study was to identify thermometer usage behaviors through different means of data collection.

Methods: A food safety lesson on minimum internal temperature and correct thermometer usage was taught in high school family and consumer sciences classes before Thanksgiving break. As homework students inputted data into a web-based form on thermometer usage and endpoint temperatures. Students were asked for picture evidence of the turkey. If a photo was not provided, they were asked how they knew it was done. Results were coded, interpreted, and compared to a broader population from the International Food Information Council's (IFIC) 2016 Food and Health Survey.

Results: Forty-five of 57 (79%) of respondents used a thermometer for their turkey. Four types of thermometers were used: dial (*n*=21), pop-up (*n*=13), digital (*n*=11), liquid (*n*=1), and some were undetermined (*n*=2.) Of respondents, 31% (*n*=18) had a minimal internal temperature of 165°F, 7% (*n*=4) were below 165°F, 21% (*n*=12) between 165 to 180°F, and 21% (*n*=12) were undetermined. Respondents provided pictures to show different thermometer placements for measuring, where 30% (*n*=17) placed it in the breast, 21% (*n*=12) in the thigh, and 5% (*n*=3) were undetermined.

Significance: There is a high usage of thermometers compared to IFIC's data on use. The data suggests the viability of citizen science in classrooms as an intervention method and a way to collect data, effectively, using pictures by providing interactions for participants with food safety information and using a primary source of information rather than self-reported data, respectively.

T3-11 Valuable Metrics That Link Training to Successful Implementation

ZHENGFANG WANG and Janie Dubois
University of Maryland - FDA JIFSAN, College Park, MD

Introduction: As Produce Safety Standards under the U.S. Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA) evolve, the global food industry must ensure food handlers, at all levels, receive timely and pertinent education and training to stay ahead of potential problems. As an established training provider, the Joint Institute for Food Safety and Applied Nutrition (JIFSAN) has achieved positive effects through essential courses and workshops regarding food safety, risk analysis in general, and global food law and regulation.

Purpose: As companies and consulting firms targeting the training needs associated with food safety, and more specifically, FSMA are being created in great numbers, we wish to share success stories of over 3,350 trainees from about 55 countries.

Methods: We have implemented effective metrics for measuring values of education and training programs to drive continuous improvements, including program sustainability, instructor evaluation, knowledge measurement before and after training, learner satisfaction, and end-user behavior change. A partnership between the University of Maryland, the FDA, and the private sector has provided us with resources to develop and sustain fit-for-purpose training programs. Collaborations have, also, extended to include federal and international government agencies, industry, academic institutions, and consumer groups.

Results: We will discuss how JIFSAN has built programs through partnerships to promote food safety at home and abroad, leverage and share resources, create a neutral environment conducive to exchange of ideas and research, and develop domestic and international collaborations. Examples of several past projects will be provided to illustrate pitfalls and suggest metrics monitoring to improve outcome.

Significance: Our valuable metrics that link training to successful implementation will definitely help the audience to advance education programs of their own.

T3-12 Capacity Building through Water Quality and Safety Analyses in Herat, Afghanistan

Paul Ebner[1], Amanda Deering[1], Mosa Mojadady[2], Zahra Rahimi[2], Roma Amini[2], Nesar Isaqzehi[2], Ehsanulla Azizi[2], Ershad Ershad[2], Solaiman Barak[2], Maqsood Popal[2], Shakib Amini[2], Neman Mohammadi[2], Mirwais Rahimi[1], Kevin McNamara[1] and **HALEY OLIVER**[1]
[1]Purdue University, West Lafayette, IN, [2]Herat University, Herat City, Afghanistan

Introduction: Contaminated water is a leading cause of approximately 600 million annual cases of foodborne disease, globally. Twenty percent of child mortality in Afghanistan is attributed to diarrheal disease. There are limited data on water quality and safety in Afghanistan, as well as limited laboratory capacity for food and water analyses.

Purpose: The purposes of this study were to train the first class of food technology undergraduate students at Herat University (Herat City, Afghanistan) in basic water quality and safety laboratory techniques, while concurrently conducting a regional water assessment study.

Methods: A total of 236 water samples from private wells (*n*=128) and taps from the municipal water system (*n*=108) were collected from Herat Province, Afghanistan. Samples were aseptically collected, transferred, and analyzed at the Herat University Food Technology Laboratory. Nitrate, nitrite, lead, phosphate, and arsenic concentrations were determined by spectrophotometry and water hardness by titration. Coliform and generic *Escherichia coli* concentration were determined by filtration.

Results: We did not detect arsenic (0 of 236) in any of the samples. All samples tested for lead (*n*=28) were below EPA maximum contaminant levels (MCL) (ave. 3.2 µg/liter). In contrast, 28 of 233 samples had nitrate levels greater than the MCL (10mg/liter) and 15 of 235 samples had nitrite levels >300µg/liter. On average, well water was harder than tap water; there were no differences in phosphate levels. Further, 92 of 213 (43.2%) had detectable coliforms (ave. 19.3 CFU/100 ml) and 52 of 215 (24.2%) had detectable *E. coli* (ave. 28.6 CFU/100 ml). *Escherichia coli* was detected in 21.2% and 26.7% of tap and well water, respectively.

Significance: This study indicated clear needs for systematic analyses of Herat City water to develop plans for water quality improvement and management. Engaging the students in research projects teaches basic research and analytical skills needed in the economy to address endemic water and foodborne diseases.

T4-01 Validation of a Multiplex Real-time PCR Method for the Detection of Crustacean Allergens (Shrimp, Crab, and Lobster) in Complex Food Matrices

SARAH STADIG and Anne Eischeid
U.S. Food and Drug Administration, College Park, MD

Introduction: Crustacean shellfish are identified as one of the eight major allergenic foods and food groups in the Food Allergen Labeling and Consumer Protection Act (FALCPA) and affect approximately 2% of the American population. FALCPA requires that different types of crustacean shellfish (shrimp, crab, and lobster) must be differentiated on food labels. While ELISA methods are unable to distinguish between these different crustacean species, PCR-based detection methods have been shown to detect and differentiate all three.

Purpose: Previously, our laboratory had validated individual real-time PCR assays for shrimp, crab, and lobster. Here, we describe a multiplex method for the detection of all three crustacean types in one assay.

Methods: Assays targeting the 12S gene were previously validated for shrimp, crab, and lobster. The probes were labeled with separate fluorophores for shrimp, crab, and lobster and the master mix was prepared with all primers and probes in the same reaction. The method was evaluated in complex food matrices for the detection and differentiation of crustacean type.

Results: Assays individually validated for probe performance produced linear standard curves with efficiencies in the range of 86.8 to 91.6% and R2 values between 0.997 to 0.999. The multiplex method produced linear standard curves for each target within appropriate efficiency range 94.6 to 111.7% and R2 values within appropriate range 0.980 to 0.995. There were no adverse effects from the cooccurring assays. All assays were evaluated from 0.1 ppm to 106ppm DNA equivalence in food matrices.

Significance: This multiplex method allowed for a more efficient way to detect and identify the crustacean allergen or multiple allergens in one assay and will be used for direct comparison with ELISA detection methods.

T4-02 Comparison of Methods for the Detection and Isolation of Shiga Toxin-producing *Escherichia coli* in Meat Samples

Mst. Thangima Zannat[1], Carlos Leon-Velarde[1], Saleema Saleh-Lakha[1], Jiping Li[1], Honghong Li[1], Anli Gao[1], Roger Johnson[2] and **SHU CHEN**[1]
[1]University of Guelph, Guelph, ON, Canada, [2]National Microbiology Laboratory at Guelph, Guelph, ON, Canada

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are an important cause of foodborne illness in humans and can result in hemolytic uremic syndrome (HUS). Certain STEC serotypes have increased potential in being implicated in foodborne outbreaks.

Purpose: The objective of this study was to compare the PCR-Rainbow agar (PCR-RB) method to a PCR-immunoblot (PCR-IB) method for the detection and isolation of STEC from meat samples.

Methods: Retail meat samples, including raw meat (ground beef, pork sausages, pork kabobs) and ready-to-eat (RTE) meat (salami, pepperoni, summer sausages) were acquired and inoculated with STEC strains of O26, O45, O103, O11, O121, O145, and O157, at low (1 to 5 CFU/25g) and high levels (10 to 50 CFU/25g). Samples were enriched in modified tryptone soya broth (supplemented with vancomycin and cefsulodin after 4 h enrichment time) for a total of 20 to 24 h at 42±0.5°C and assayed via PCR-RB and PCR-IB. All inoculated samples were subjected to culture isolation regardless of the PCR result.

Results: For RTE meat samples (*n*=48), both PCR-RB and PCR-IB were equally effective in screening and isolating STEC. In total, 12 of 20 low and 23 of 23 high inoculated samples were positive, achieving 100% sensitivity, 100% efficacy, and 0% false negative rate. For raw meat samples (*n*=48), the PCR screening had 100% agreement between the two methods in identifying 15 of 20 low and 23 of 23 high inoculated samples. The immunoblot isolation procedure, however, was more effective in isolating STEC from raw meat (100% sensitivity, 100% efficacy, and 0% false negative rate) compared to the PCR-RB isolation procedure (78.9% sensitivity, 83.3% efficacy, and 21.2% false negative rate).

Significance: The results of this study demonstrated that the immunoblot isolation procedure can be considered an effective alternative to standard direct plating methods for isolating STEC from potentially contaminated meat samples.

T4-03 Novel Immunoassay Pathogen Detection Method for *Listeria* spp. in Food and Environmental Samples

SIMON ILLINGWORTH and Nevin Perera
Solus Scientific Solutions Ltd, MANSFIELD, United Kingdom

Introduction: Immunoassay-based pathogen detection methods are typically comprised of two selective enrichment steps prior to detection, resulting in a longer time to result than some alternative methods. We have developed a next generation *Listeria* spp. immunoassay, which provides negative or presumptive positive results within 26 to 29 hours.

Purpose: The aim of our study was to shorten the total method time of our existing Solus *Listeria* immunoassay by improving the enrichment and immunoassay performances.

Methods: The resulting *Listeria* immunoassay was performed following enrichment of a 25 g sample in 225 ml selective broth for 26 hours at 30°C. Analytical sensitivity was determined with enumerated heat-killed cultures at 5×10^5, 1×10^4, and 1×10^6 CFU/ml. Inclusivity and exclusivity was tested with 10 *Listeria* spp. and 38 non-*Listeria* spp. Samples spiked at 1 to 10 CFU *Listeria* per 25 g. Detection of the target organism was tested within naturally contaminated matrices (ready-to-eat and ready-to-reheat, meat, dairy, vegetable and seafood products, ingredients, feed products and environmental samples).

Results: The analytical sensitivity of the assay was calculated to be approximately 10^4 to 10^5 CFU/ml. All food associated *Listeria* spp. were detected and a panel of 38 non-*Listeria* spp. gave negative results indicating satisfactory inclusivity and exclusivity. Three hundred and one naturally contaminated food samples were tested from the categories above. Sixty-four samples were found to contain *Listeria* by the EN ISO 11290 reference method and 62 of 64 samples were positive by the immunoassay and subsequent confirmation from the alternative enrichment protocol. No false positive results were observed.

Significance: The resulting immunoassay retains the testing efficiency benefits of the original assay, which, when used in conjunction with liquid handling automation, enables processing of large numbers of samples. Increased sensitivity enables a faster time to result, which is clearly beneficial for rapid turnaround of food and environmental test samples when a contamination source needs to be identified and eliminated.

T4-04 Determination of Trace Metals in Several Off-the-Shelf Spices Using Aerosol Phase Dilution

NEAL JULIEN

MRIGlobal, Palm Bay, FL

Introduction: In 2016 the FDA issued a nationwide recall for several commercially available turmeric powders due to elevated levels of lead. Turmeric and other spices are widely used in many cuisines, and as several cultures make extensive use of selected spices, either in powdered or natural form, exposure to lead and other trace metals by consuming foods prepared with these spices is a concern.

Purpose: Our laboratory recently analyzed 11 commercially available spices for trace metals to evaluate aerosol phase dilution for contamination control and lower detection limits.

Methods: Two different brands of spice powders (except annatto powder) were purchased at a local supermarket, prepared in triplicate by hot acid digestion using a mixture of nitric acid and hydrochloric acid, then analyzed by collision cell ICP-MS for arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), thallium (Tl), and zinc (Zn). The spices analyzed were annatto powder, cinnamon powder, curry powder, paprika, smoked paprika, and turmeric powder. NIST SRM-1647 (Peach Leaves) was used as a control and standard quality control procedures were followed during the analysis.

Results: Results for the NIST sample were within ±10% of the expected values and all other QA/QC measurements were within target specifications. Measured concentrations were as follows (detection limit, range) - As: 0.007, 0.010 to 0.200 µg/g; Cd: 0.001, 0.012 to 0.294 µg/g; Cr: 0.057, 0.158 to 4.32 µg/g; Cu: 0.021, 1.52 to 13.6 µg/g; Pb: 0.036, 0.051 to 0.470; Mn: 0.041, 9.40 to 168; Tl: 0.001, 0.001 to 0.022 µg/g; Zn: 1.43, 8.04 to 29.1 µg/g.

Significance: Aerosol phase dilution allowed digested samples to be prepared in a low final volume, facilitating lower detection limits while reducing the potential for sample contamination from additional sample handling. While differences by brand were observed, these results indicate exposure to trace metals by consumption of foods prepared using these spices is low, but elements with nutritional value were present in significant concentrations.

T4-05 Bacteriophage-based Dipstick: Inkjet Printing of Bacteriophages to Detect Different Foodborne Pathogens

HANY ANANY[1], Jennifer Sohar[2], Heather Fenn[2], Noha Eldougdoug[3], Nada Alasiri[2], Luba Brovko[4] and Mansel Griffiths[5]

[1]Agriculture and Agri-Food Canada, Guelph, ON, Canada, [2]University of Guelph, Food Science Department, Guelph, ON, Canada, [3]Microbiology Department, Benha University, Benha, Egypt, [4]University of Guelph, Food Science Department, Guelph, ON, Egypt, [5]University of Guelph, Guelph, ON, Canada

Introduction: Foodborne pathogens continue to burden our economy and society with illness. Therefore, the rapid detection of foodborne pathogens is vital to a safe and secure food supply. Bacteriophages (phages) are viruses capable of infecting and replicating within bacteria in a strain-specific manor. The ubiquitous and selective nature of phages makes them ideal for the detection and biocontrol of bacteria.

Purpose: The objective of this research was to develop and test two phage based biosensors for the detection of *Escherichia coli* O157:H7, *Escherichia coli* O45:H2 and *Salmonella* Newport from inoculated spinach, ground beef, and chicken homogenates, respectively.

Methods: Both detection methods use a phage-based amplification approach followed by real-time PCR to detect *E. coli* in only eight hours. The first method used phage immobilized to modified paramagnetic silica beads that can be mixed with the food sample. The second sensor was developed as a paper dipstick by printing phage onto commercial paper with a piezoelectric printer. This phage dipstick was used to capture and infect the host bacteria from inoculated food samples.

Results: The detection limit was found to be 10^2 to 10^3 CFU/ml for phage immobilized on the modified beads in spinach homogenate and 10 CFU/ml and 10^3 for phage immobilized on paper (dipstick) in spinach homogenate and ground beef homogenate, respectively. Two other lytic phages against *E. coli* O45 and *Salmonella* Newport were printed on ColorLok paper and the developed bioactive paper was used to detect target bacterial strains in broth and meat samples. The detection limit was found to be around 10 CFU/ml in broth and around 50 CFU/ml in ground beef and chicken homogenates, for both targets, within less than eight hours.

Significance: This detection method is rapid, cost-effective, and can be applied to a broad range of foodborne pathogens.

T4-06 Development of a Novel Hygiene Monitoring System Based on the Detection of Total Adenylate

MIKIO BAKKE[1] and Shigeya Suzuki[2]

[1]Kikkoman Biochemifa Company, Noda-shi, Chiba, Japan, [2]Kikkoman Corporation, Noda-shi, Chiba, Japan

Introduction: ATP is the universal energy molecule found in animals, plants, and microorganisms. ATP rapid hygiene monitoring tests have been employed in food industry to ensure adequate cleanliness is being maintained. However, since ATP is hydrolyzed to ADP and AMP by metabolic processes, heat treatment, or in acidic/alkaline conditions, the components of total adenylate (ATP+ADP+AMP; A3) could be more reliable indicators of lack of cleanliness derived from food residues, which may cause bacterial growth and allergen contamination.

Purpose: In this study, a novel enzymatic method to measure A3 was developed. Furthermore, its significance was verified by comparing the amount of A3 and ATP in various food samples.

Methods: ATP was measured by luciferin-luciferase assay, which produces luminescence and AMP. A3 measurement was based on luciferin-luciferase assay with pyruvate kinase and pyruvate phosphate dikinase, which can convert ADP into ATP and recycle AMP into ATP in the presence of phosphoenolpyruvic acid, respectively. Food samples were blended with water and then processed by centrifugation. The supernatants were diluted with water and applied to the assay. The assays were performed in triplicate. A3 and ATP were quantified by measuring luminescence calibrated against known amounts of standards.

Results: The newly developed A3 assay system afforded equivalent linear calibration curves between relative light units and the amounts of ATP, ADP, and AMP, respectively. The amounts of A3 were more than two orders of magnitude greater than that of ATP alone in various foods, such as meat, seafood, whole egg, dairy, beans and nuts. For example, A3 and ATP (pmol/g) in raw chicken were 2.6×10^4 (3% CV) and 3.2×10^1(10% CV), respectively.

Significance: The A3 assay was developed and appeared to be more suitable for detection of food residues, which potentially cause bacterial growth and allergen contamination, than the conventional ATP assay.

T4-07 Extended Enrichment Procedures Can be Used to Define False Negative Rate for Cultural Gold Standard Methods for *Salmonella* Detection Facilitating Comparisons between Gold Standard and Alternative Methods

GENEVIEVE SULLIVAN[1], Xiaodong Guo[1], Jeffrey Tokman[1], Sherry Roof[1], Aljosa Trmcic[1], Robert Baker[2], Silin Tang[2], Peter Markwell[2], Martin Wiedmann[1] and Jasna Kovak[1]

[1]Cornell University, Ithaca, NY, [2]Mars Global Food Safety Center, Beijing, China

❖ ***Developing Scientist Competitor***

Introduction: Evaluation of alternative detection methods for foodborne pathogen (e.g., rapid methods), typically involves comparisons of an alternative method against a gold standard culture method. In these evaluations, it is often assumed that the gold standard does not show false negative results, however, this is not likely correct.

Purpose: We, thus, employed a strategy that uses extended enrichment times to evaluate a gold standard method for detection of *Salmonella* (i.e., the FDA BAM method) in dry pet food inoculated at fractional positive levels.

Methods: This evaluation was performed with five *Salmonella* strains (each tested in 20 replicates) that were selected due to their poor ability to grow in enrichment media and to be detected by the rapid detection method.

Results: Four samples that were negative with the standard BAM method, but positive with the extended BAM method (for example after primary enrichment of 72 h rather that the standard 24 h). These data indicated a false negative rate, determined by the BAM method, to be 7%. By comparison, previously evaluated alternative methods showed false negative rates as low as 4%. With the new data presented here, we can now conclude that some of these previously evaluated assays showed lower or comparable false negative rates relative to the BAM method. Additional rapid methods for detection of *Salmonella* in dry pet food were conducted and some methods showed false negative rates in excess of 82%.

Significance: Overall, our data indicated that further stringent evaluation of standard culture-based methods will be valuable for determining the efficacy of alternative (e.g., rapid) detection methods. There still are commercial methods that produce substantial false negative rates that are clearly inferior to standard culture-based detection methods.

T4-08 Digging Deep: Making the Case for Molecular Based Detection with Real-world Performance and Discrepant Evaluation

WILLIAM CHANEY[1], Sarah Verver[1], Janelle Lauffer[1], Cambria Berry[1], Ted Andrew[2] and Mary Duseau[2]

[1]Roka Bioscience, San Diego, CA, [2]Roka Bioscience, Warren, NJ

Introduction: Alternative molecular based detection technologies are rapidly evolving, yet results from such methods are often questioned or dismissed based upon follow up with imperfect culture based methodologies.

Purpose: This study evaluated the performance of the Atlas® *Listeria* Environmental (LE) Detection Assay for *Listeria* spp. detection in real-world environmental samples with investigation of initially discrepant results.

Methods: Environmental sponge swabs (n≥700) were collected from zones 3 and 4 of multiple processing environments. Swab samples were enriched with 90 ml of Actero *Listeria* Enrichment Media for 24 hours at 35°C prior to assaying with the LE assay using two analytical volumes, 200 µl and 2,000 µl. Screen positive results were initially struck to Modified Oxford Agar and transferred to Fraser secondary enrichment with subsequent plating to MOX and *Listeria* Chromogenic agars. Absence of typical morphology resulted in further analytical and culture analyses including PCR, IMS, and Filtration methodologies. Purified isolates were identified with biochemical or sequencing methodologies.

Results: Of *n*=750 samples, the LE assay reported 24 presumptive results for both the 200 and 2,000 µL analytical volumes. Of the 24 screen positive samples, *Listeria* spp. was isolated from 23 with standard or follow up culture analyses. Two discrepant samples were resolved utilizing a modified sample preparation for removal of free nucleic acid. In two cases, a biochemical method failed to identify typical isolates, which were identified as *L. monocytogenes* and *L. innocua* by 16S based identification. Other samples required extensive culture attempts to obtain isolates for microbial identification, which would be impractical under current industry testing scenarios and turnaround times.

Significance: These real-world data supported the molecular based detection of *Listeria* spp. while highlighting potential pitfalls and shortcomings of downstream confirmation processes.

T4-09 Insect Contaminants in Foods: Detection Limits of a Qualitative PCR-based Method

MONICA PAVA-RIPOLL, Amy K. Miller and George C. Ziobrio

U.S. Food and Drug Administration-CFSAN-OFS, College Park, MD

Introduction: Macroscopic and microscopic methods are currently used in assessing insect contaminants in foods. Sample analyses with these methods are time consuming and require analysts with high degrees of additional training and skills. Molecular based methods for screening of microbial and chemical food contaminants are being rapidly adopted and may be useful in the detection of insect contaminants in foods.

Purpose: This study determined the detection limits of insect fragments in whole wheat flour using singleplex and multiplex qualitative endpoint PCR.

Methods: Fragments of three known vectors of foodborne pathogens (the housefly, *Musca domestica*; the American cockroach, *Periplaneta americana*; and the pharaoh ant, *Monomorium pharaonis*) and two food storage insect pests (the Indian meal moth, *Plodia interpunctella*, and the red flour beetle, *Tribolium castaneum*) were added to whole wheat flour at spiking levels of 1, 0.1, 0.01, and 0.001%. Flour without added insect fragments was used as the control. Using custom primers and endpoint PCR, the extracted gDNA was used as template to amplify fragments of the protein-encoding wingless (*wg*) gene and the cytochrome oxidase I (*COI*) gene. The visualization of amplicons of expected sizes was considered positive for the presence of insects.

Results: DNA was successfully isolated from all samples. The *COI* primers amplified a ~150 to180 bp fragment from *M. domestica*, *P. interpunctella* and *T. castaneum*, whereas the *wg* primers amplified a ~410 to 430 bp fragment from *P. americana* and *M. pharaonis*. The intensity of an amplicon band was positively correlated to the spiking level. Insect fragments were detected at spiked levels as low as 0.001% (10 ppm).

Significance: Additional multiplex results will be discussed, and together, these results suggest that molecular methods can potentially be used for the rapid detection of insect contaminants in foods.

T4-10 Culture-independent Detection and Confirmation of Shiga Toxin-producing *Escherichia coli* by Digital PCR

JIANFA BAI, Xuming Liu, Lance Noll, Xiaorong Shi, T G Nagaraja and Gary Anderson
Kansas State University, Manhattan, KS

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are major foodborne pathogens. None of the current PCR assays can confirm whether a detected virulence gene is associated with a particular serogroup of STECs from complex matrices. The current USDA-FSIS recommended method takes one week to result due to confirmation through cultural isolation; this does not fit high throughput settings.

Purpose: This study was undertaken to develop a digital PCR assay for the detection and confirmation of major STECs without cultural isolation.

Methods: Seven major STEC serogroups, O26, O45, O103, O111, O121, O145, and O157, were used as pure cultures and to inoculate cattle feces with three replications. For each serogroup, a strain that carried both the serogroup-encoding gene and an *stx* gene was compared to a mixture of two strains (each carried one of the two genes) using Thermo Fisher QuantStudio 3D and Fluidigm Biomark digital PCR systems. The assay was also evaluated with 100 cattle fecal samples.

Results: The gene association rates (GAR, percentage of dual signals) by QuantStudio 3D for the O157 strain that carried O157-antigen gene and *stx*2 was 62.5% for culture and 77.2% for spiked feces; GAR for the mixture of two strains was 21.2% for culture and 28.3% for spiked feces. GARs by Fluidigm Biomark for each of the seven major STECs that carried two genes ranged 67.3 to 90.5% for culture and 74.6 to 88.0% for culture-spiked fecal samples. GARs for the two genes carried by separate genomes were between 8.7 and 28.8% for culture, and 8.3 to 30.9% for culture-spiked fecal samples. A strain carrying three genes (*rfb*E-O157, *stx*2, and *eae*) was correctly identified. Three O103 strains that carried *eae* and three O45 strains that carried *stx*1 genes were identified from a collection of 100 cattle fecal samples.

Significance: Our digital PCR assay is a culture-independent and high throughput assay. It is capable of detecting STECs in only two days.

T4-11 Integration and Public Health Protection as Outcomes of Food Laboratory Accreditation

Shari Shea and **ROBYN RANDOLPH**
Association of Public Health Laboratories, Silver Spring, MD

Introduction: FDA has invested over $50 million towards the accreditation of state laboratories to the ISO/IEC 17025:2005 standard. Accreditation is an integral part of mutual reliance and a critical element of an Integrated Food Safety System (IFSS). A fully functioning IFSS allows for acceptance of state data in federal enforcement actions and means FDA and states can act efficiently in the prevention of foodborne illnesses.

Purpose: This session will describe the impact of collaborative efforts by FDA, the Association of Public Health Laboratories (APHL), the Association of Food and Drug Officials (AFDO), and the Association of American Feed Control Officials (AAFCO) to promote laboratory accreditation for the nation's food and feed laboratories.

Methods: In 2012, FDA awarded a cooperative agreement to APHL, AFDO, and AAFCO to facilitate long-term improvements to the national food and feed safety system by supporting laboratories seeking ISO 17025 accreditation. That year, FDA also funded 31 laboratories that perform food testing for state regulatory programs to achieve ISO accreditation. In 2015, six more food laboratories and 20 feed laboratories received ISO funding. In 2016, FDA funded an additional feed laboratory. Additionally, a structured accreditation support program was established within FDA to provide guidance and technical assistance to the funded laboratories.

Results: As of Oct 2016, 22 FDA-funded laboratories have either achieved or expanded their scopes of accreditation. Active monitoring of cooperative agreement deliverables shows that the original FDA-funded food laboratories are on track to achieve or expand their accreditations by August 2017. As direct results of accreditation and the development of sampling plans required by the ISO grants, specific success stories from several states will be presented.

Significance: Investment in laboratory accreditation helps protect public health by ensuring that regulatory agencies can act quickly on high quality analytical data from accredited laboratories when those laboratories detect a problem in the food supply. Additionally, routine food product sampling agreements have proven very valuable and effective.

T4-12 A Unique Workflow Consisting of Metagenomic Sequencing and Bioinformatic Analysis to Routinely Recover High Quality *Cyclospora cayetanensis* Whole Genome Sequences from Clinical Samples

GOPAL GOPINATH[1], Hediye Cinar[2], Helen Murphy[2], ChaeYoon Lee[1], Sonia Almeria[2], Mauricio Durigan[2] and Alexandre da Silva[2]
[1]U.S. Food and Drug Administration, Laurel, MD, [2]U.S. Food and Drug Administration–CFSAN, Office of Applied Research and Safety Assessment, Laurel, MD

Introduction: The foodborne coccidian parasite Cyclospora cayetanensis causes endemic and epidemic cyclosporiasis. Lack of molecular epidemiological tools hampers detection and strain identification of this organism in the food supply.

Purpose: In this technical session, a workflow to generate good quality assemblies of *Cyclospora*genomes by deep sequencing of clinical samples is presented.

Methods: Total DNA from a Nepalese fecal sample, NF1, was extracted from isolated oocysts. Nextera, Nextera XT, and Ovation kits were used to prepare libraries for metagenomic sequencing on an Illumina Miseq platform. Bowtie2, CLC genome bench, Geneious, MEGA7, perl scripts, AUGUSTUS, Metaphlan2, and Companion were used for bioinformatic analysis of HCNY and HEN01 genome datasets, which were obtained from NCBI. *Eimeria necatrix* was used as a reference for gene prediction training. *Apicomplexan*genomes were obtained from NCBI.

Results: Ovation libraries with insert sizes from 800 to 1,000 bases had higher coverage of *Cyclospora* reads. *Cyclospora cayetanensis* HCNY WGS assembly (44 MB) was used for mapping metagenomic reads. Metaphlan2 analysis showed negligible bacterial contamination in the mapped reads, which were trimmed and assembled to generate a 42.3 MB WGS assembly with 1,786 contigs. The AUGUSTUS program predicted 7,402 and 8,037 proteins, respectively, from NF1 and HCNY. Approximately 5,000 homologs of *Eimeria* were identified in both *Cyclospora* genomes. Complete apicoplast and mitochondrial genomes recovered from these assemblies were identical to the reference genomes. Strain level differences between NF1, HCNY, and HEN01 assemblies were obtained by allele detection in exons. Evolutionary analysis of NF1 *Cyclospora* proteins with other *Eucoccidioridan apicomplexans*confirmed a divergent phylogenetic relationship among these important parasites.

Significance: The presented workflow to recover WGS assemblies of *C. cayetanensis* from fecal samples will enable the availability of more genomes for the food safety community. Larger allelic data from *C. cayetanensis* genes will facilitate the development of molecular fingerprinting tools for source-tracking of this important foodborne parasite.

T5-01 Application of Edible Coatings Formulated with Antimicrobials to Control *Listeria monocytogenes* as Surface Contaminants on Fresh Cheese

STEPHANIE BARNES, Sarah Kozak and Dennis D'Amico
University of Connecticut, Department of Animal Science, Storrs, CT

❖ ***Developing Scientist Competitor***

Introduction: Despite efforts to control *Listeria monocytogenes* (Lm) in dairy processing environments, contamination and subsequent outbreaks of listeriosis continue to occur. The ability of Lm to grow during refrigerated storage necessitates strategies to prevent contamination, reduce pathogen numbers, and limit growth.

Purpose: The objective of this study was to determine the efficacy of edible antimicrobial coatings to control *Lm* on fresh cheese (Queso Fresco) applied before (precoated; PC) or after (preinoculated; PI) surface contamination.

Methods: Coating solutions were formulated to contain 2% chitosan and either 5% hydrogen peroxide (HP), 5% lauric arginate (LAE), 10% sodium caprylate (SC), 25% acidified calcium sulfate (ACS), or combinations of SC with either LAE or ACS at pH ~4.5. Fresh cheese samples (25 g) were inoculated with *Lm* (10^4 CFU/g) prior to or following coating application, vacuum packaged, and enumerated, weekly, throughout storage at 7°C for 35 days.

Results: In general, there was no effect of coating application type (PI vs. PC) on *Lm* counts for all treatments except LAE+SC combinations (P=0.0002). There was a significant effect of chitosan coating, alone, on *Lm* counts for both applications when compared to control over time (P<0.0001), reaching >7 log CFU/g after 35 days. Overall, HP was the most effective bactericidal coating with counts <1 log CFU/g after 35 days. Although the effect of singular treatment of LAE did not differ from chitosan alone, singular treatments with ACS (P≤0.021) as well as combination treatments of both compounds with SC resulted in significantly lower *Lm* counts over time (P≤0.0026). Both LAE+SC coating applications were bacteriostatic while counts in both ACS+SC applications were ~5 log CFU/g at 35 days.

Significance: The identification of bactericidal and bacteriostatic edible antimicrobial coating applications that are effective when applied before or after contamination events provides a new tool for the control of *Lm* on fresh cheese for enhanced food protection.

T5-02 Characterization of Cattle Feedlot Isolated *Salmonella* spp. Bacteriophages and Evaluation of Their Antimicrobial Capacity against *Salmonella* on a Cattle Hide Model

YICHENG XIE[1] and Jason Gill[2]
[1]Texas A&M University, College Station, TX, [2]Texas A&M University, Bryan, TX

❖ ***Developing Scientist Competitor***

Introduction: Asymptomatic *Salmonella* carriage in beef cattle is a significant food safety concern; and the beef feedlot environment and the animal hide are reservoirs of this pathogen. Bacteriophages may play a role in suppressing *Salmonella* in the feedlot environment and may also prove useful as a means of controlling this pathogen in ground beef.

Purpose: The goals of this investigation were to characterize the bacteriophages and evaluatate the use of individual and mixed bacteriophages for control of *Salmonella* in a cattle hide model.

Methods: Bacteriophages targeting various *Salmonella* serovars were isolated from three cattle feedlots located in south Texas and waste water in Texas. Bacteriophage genomes were sequenced and morphology was observed via TEM. The phage host range was tested against a panel of 20 *Salmonella* isolates by spot dilution tests. The ability of phages to inhibit bacterial growth was assessed in a 96-well microtiter plate assay. Cattle hides obtained at harvest were inoculated with a cattle feedlot isolated *Salmonella* strain and treated with single and mixed bacteriophages. The treated cattle hides were homogenized and plated to observe bacterial reduction.

Results: Bacteriophages were determined to be members of the virulent Chi-like, 9NA-like, and T5-like groups of *Siphoviridae*; with one phage belonging to a novel jumbo *Myoviridae* phage type. The host ranges of phages were highly variable, with the broadest host range phage infecting 16 of the 20 *Salmonella* strains tested. Certain phage combinations were found to be able to suppress the regrowth of phage-resistant bacteria in the microtiter plate assays. *Salmonella* was significantly reduced by some single and mixed phage treatments (n=3, P<0.05), with reductions up to 1.7 log CFU/cm^2.

Significance: In this study, the ability of phages to reduce *Salmonella* on cattle hides suggested a novel means of suppressing transmission of *Salmonella* into the lymph nodes of cattle; and, thus, may be a use for controlling *Salmonella* in ground beef.

T5-03 Reduced *Campylobacter jejuni* Colonization in Poultry Ceca with Natural Phenolics from Industry Byproducts

SERAJUS SALAHEEN, Zajeba Tabashsum and Debabrata Biswas
University of Maryland, College Park, MD

❖ ***Developing Scientist Competitor***

Introduction: *Campylobacter jejuni*, one of the prominent causes of acute gastroenteritis in humans, occurs through consumption of raw and undercooked poultry. Bioactive phenolics from blackberry (Rubus fruticosus) and blueberry (Vaccinium corymbosum) pomaces can be potential antimicrobials against C. jejuni.

Purpose: This study was conducted to investigate the effect of berry phenolic extract (BPE) as water supplement on the colonization of *C. jejuni* in poultry cecum and in modulation of the gut microbiome.

Methods: A total of 120 one-day-old chicks were infected with a marked *C. jejuni* RMKm strain for 21 days, in duplicate trials, to determine effect of BPE on *C. jejuni* colonization. In a separate experiment, 200 one-day-old chicks were used for 42 days, in duplicate trials, to determine the effect of BPE on natural colonization of *C. jejuni* and modulation of the cecal microbiome by shotgun sequencing.

Results: A kanamycin resistance cassette obtained from *Escherichia coli* DH5α plasmid $p^{jet1.1}$ was inserted into the genome of *C. jejuni* RM1221 and eight logs of this marked strain (*C. jejuni* RMKm) were used to infect day-old chicks. We observed that 1.0 g Gallic Acid Equivalent (GAE)/liter of BPE reduced *C. jejuni* colonization by one log in broiler cecum compared to the control group. Natural transmission of *C. jejuni* RMKm from chicks to drinking water was observed with a contamination level of two to five logs without BPE, but none in presence of 1.0 g GAE/liter of BPE. In the natural colonization determination experiment, we observed 1.0 g GAE/liter of BPE reduced natural colonization of *Campylobacter* by two logs in cecum compared to controls. BPE supplementation was associated with altered bacterial, DNA viral, and archaeal communities, as well as functional genes and resistomes of the chicken ceca.

Significance: This study showed that bioactive extracts from berry pomace can serve as a potential alternative to synthetic antimicrobials and reduce *C. jejuni* colonization in farm animals, specifically poultry, to improve product safety.

T5-04 Use of *Olea europaea* Byproducts to Stimulate the Growth of Probiotic and Competitively Exclude Enteric Pathogens

MENGFEI PENG and Debabrata Biswas
University of Maryland, College Park, MD

Introduction: Due to the emergence of multidrug-resistant pathogens, specifically enteric bacterial pathogens, the number of effective antibiotics has drastically declined, making alternative antimicrobials essential. Olea europaea is rich in bioactive phenolic compounds such as hydroxytyrosol (HT) and oleuropein (OP), which are associated with multiple promising pharmacological activities including antioxidant, antimicrobial, and antiinflammatory activities.

Purpose: The purpose of this study was to evaluate the role of HT and OP on growth promotion of probiotic strains and their antimicrobial effect against growth and virulent gene expression of major foodborne pathogens.

Methods: The minimum bactericidal/inhibitory concentration (MBC/MIC) of HT and OP on enterohemorrhagic *Escherichia coli* (EHEC) and *Salmonella enterica* serovar Typhimurium (ST) were determined by broth microdilution method. The effects of HT and OP on *Lactobacillus casei*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum* or against EHEC and ST were investigated in mixed liquid culture conditions. Several invasion-related virulence genes of ST and EHEC, under stress of HT and OP, were also evaluated by qPCR.

Results: OP, up to 2.0% (w/v), significantly stimulated the growth of all three *Lactobacillus* strains in a dose-dependent manner, whereas only lower concentrations of HT, up to 0.2%, slightly promoted the growth of *L. rhamnosus.* In mixed culture condition, 0.05 and 0.2% HT competitively excluded both EHEC and ST within 24 h. Similarly, 1.0 and 2.0% OP completely excluded both EHEC and ST within 24 h. Further, HT significantly (P<0.05) down-regulated the expression of multiple invasion-related virulence genes such as *hilA*/*C*/*D*, *invA*/*C*/*F*/*G*/*H*, *sipA*, and *sirA*/*B* for ST and *eaeA*, *espA*/*B*/*D*, *ler*, and *tir* for EHEC (1.5 to 6.0 fold), respectively.

Significance: Both HT and OP could be strong alternative antimicrobials, as well as stimulators for *Lactobacillus* growth, which may serve as a gut microbiome modulator in preventing foodborne enteric infections.

T5-05 Control of *Escherichia coli* O157:H7 with Deodorized Mustard in Mennonite Fermented Sausages

ROCIO MORALES-RAYAS[1], Angela Tellez[2], Richard Holley[3], Jeffrey Farber[1] and Arthur Hill[2]
[1]University of Guelph, CRIFS, Department of Food Science, Guelph, ON, Canada, [2]University of Guelph, Guelph, ON, Canada, [3]University of Manitoba, Winnipeg, MB, Canada

Introduction: Six small processors in the Mennonite community in Southwestern, Ontario decided to explore a nonthermal alternative to control *Escherichia coli* O157:H7 in fermented sausages. Several compounds were tested to control VTEC, but deodorized mustard seemed the most promising. Deodorized mustard has shown a detrimental effect on VTEC; however, there are reports that consumers have taken issue with organoleptic qualities when used at certain concentrations.

Purpose: The purpose of the study was to examine the potential use of deodorized mustard for the control of *E. coli* O157:H7 in Mennonite dry fermented sausages.

Methods: A ranking sensory tasting was done on Menonnite sausages manufactured with and without mustard. Summer sausages were then prepared under laboratory conditions. Two fermentation temperatures were tested for meat batters that were inoculated with a five-strain cocktail of *E. coli* O157:H7 to yield approximately seven log CFU/g. The batters were manually forced into a 120 mm diameter fibrous casing to form one kilogram sausage sticks. The sausages were sealed and, then, hung on horizontal aluminum sticks and placed into a smoke chamber to continue fermentation and smoking. Sausages were dried under controlled conditions in environmental chambers.

Results: From an organoleptic standpoint, the concentration of mustard used in this study was determined based on data collected from 100 participants, which showed no significant differences in the taste of sausages prepared with and without mustard. This mustard concentration used, along with a fermentation temperature of 27°C and smoking at 29°C, led to a five-log reduction of *E. coli*O157:H7 after 21 days drying.

Significance: This study provided validation data to show that deodorized mustard can be used effectively to control *E. coli* O157:H7 in Mennonite dry fermented sausages. Once approved by Canadian regulatory authorities, the Mennonite community and other medium-size processors in Ontario will be able to use the validated process to comply with current regulations.

T5-06 The Lytic Capacity of Listeriophage is Affected by Phenotypic and Genotypic Characteristics of *Listeria monocytogenes*

AJITA SUNDARRAM, Jia Liu, Karina Desiree, Paige LeMaster, Danielle Marks, MaryKate Harrod, Shelby Meyer, Paul Ebner and Haley Oliver
Purdue University, West Lafayette, IN

❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* contamination in food processing environments is a known source of cross-contamination to final products. Due to their specificity, there is increased interest in bacteriophage as a control strategy for *L. monocytogenes*.

Purpose: The purpose of this study was to measure the spectrum of a listeriophage cocktail against a library of *L. monocytogenes* strains with varied phenotypic and genotypic characteristics.

Methods: Lytic capacity of the listeriophage was tested against 381 *L. monocytogenes* isolates representing 61 strains by standard spot assay in LB agar (supplemented with MOPS and glucose) at 21.5°C and 30°C. Lytic capacity of each isolate was evaluated on a scale of 0 to 3 (0: no lysis; 1: partial lysis; 2: ring of confluent lysis around lawn in center; 3: confluent lysis). Multinomial logistic regression was used to determine proportional odds estimates for phage susceptibility based on isolates' persistence vs. transience, PFGE type, and attachment capacity to abiotic surfaces at different temperatures and concentrations.

Results: If a *L. monocytogenes* isolate was historically persistent in an environment, the odds of its lysis score being zero, one, and two increased by 11.4, 8.0, and 3.7 times, respectively, relative to lysis score three. Similarly, for incubation temperature 21.5°C, the odds of lysis score being zero, one and two increased by 4.0, 3.5, and 2.5 times, respectively, relative to score of three. The effect of attachment capacity and concentration of phage was not significant (P>0.05) when persistence and temperature were included in the model.

Significance: This study showed that the lytic capacity of listeriophage may be reduced if *L. monocytogenes* strains have persistent phenotypes. Whether and how these characteristics impact the efficacy of phage in controlling *L. monocytogenes,* in commercial food processing environments, is the subject of further study.

T5-07 Disruption of Shiga-toxigenic *Escherichia coli* Biofilms In Vitro and on Food Contact Surfaces Using Bacteriophages

PUSHPINDER KAUR LITT, Tony Kountoupis, Pramila Lamichhane and Divya Jaroni
Oklahoma State University, Stillwater, OK

❖ ***Developing Scientist Competitor***

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are a significant public health concern. Their biofilms on food and food-contact surfaces pose a significant challenge to the food industry. These biofilms can be very difficult to penetrate, making conventional control methods insufficient. Targeted use of bacteriophages that can disrupt these biofilms could reduce this problem in the food industry.

Purpose: This study evaluates the efficacy of bacteriophages against STEC biofilms in vitro and on food contact surfaces.

Methods: Bacteriophages (n=52), isolated from cattle operations, showing lytic activity towards STEC strains were tested for biofilm-inhibiting capabilities in vitro and on food contact surfaces. Phage treatments (eight log PFU/ml) were prepared in phosphate buffered saline (PBS). Biofilm-forming STEC strains (O157, O26, O45, O103, O111, O121, O145), were used to form biofilms in microtiter plates (seven log CFU/ml), stainless steel (SS), or high density polyethylene (HDPE) coupons (nine $\log_{10}$ CFU/ml). Following biofilm formation, plates/coupons were treated with phage treatments or used as PBS-controls. For in vitro evaluation, microtiter plates were incubated six hours at 37°C. Changes in bacterial absorbance (A_{595}) were observed at zero, three, and six hours. Coupons were incubated at 37°C for 16 h and STEC survival was determined by plating onto tryptic soy agar. Data was analyzed using one-way ANOVA (P<0.05).

Results: Bacteriophages showed significant reduction (P<0.05) in STEC biofilms compared to the positive control in vitro and on food contact surfaces. A reduction in absorbance (2.262 nm at 0 h to 0.808 nm at 6 h) was observed in phage-treated wells compared to control wells, indicating STEC biofilm disruption. Of 52 phages, 62% showed STEC biofilm disruption in vitro. On SS and HDPE coupons, phage treatments showed 1.8 to 5.8 log CFU/cm^2 reduction in STEC populations, compared to the control.

Significance: Bacteriophages specific for STECs, with high lytic activity and ability to reduce biofilm formation, could be used as biological control agents in the food industry.

T5-08 Reverting Multidrug-resistant Phenotypes of *Escherichia coli* Isolated from Cattle Using 1-(1-Naphthylmethyl)-Piperazine

JOÃO ANES, Daniel Hurley, Séamus Fanning and Shabarinath Srikumar
University College Dublin, Dublin, Ireland

❖ ***Developing Scientist Competitor***

Introduction: The extensive use of antimicrobial agents in both the health and food sector has led to the emergence of multidrug resistant (MDR) bacteria, a development of importance to public health. Efflux pumps extrude antimicrobial compounds from cells contributing to the development of resistance. Chemosensitizers with the capacity to modulate efflux pump activity are being studied as adjuvants in efforts to reverse resistant phenotypes. However, little is known about their efficacy and mechanism of action.

Purpose: The purpose of this study was to systematically analyze the MDR reversal activity of the chemosensitizer 1-(1-naphthylmethyl)-piperazine (NMP), when applied as an adjuvant with antibiotics on both planktonic and sessile *Escherichia coli* cells.

Methods: Bovine *E. coli* isolates from the UCD Veterinary Hospital were screened for their MDR phenotype. A panel of 12 isolates, resistant to different classes of antibiotics including fluoroquinolones, tetracyclines, and chloramphenicol, were further studied. All were characterized by whole genome sequencing. The ability to form biofilm and fimbriae was, also, determined. Minimum inhibitory concentration (MIC) for each antibiotic, alone or in combination with NMP at sub-MIC levels, was determined by broth microdilution, using planktonic and sessile-grown cells. Transmission electron microscopy (TEM) using NMP was performed.

Results: Isolates had diverse AMR and virulence gene profiles. Based on these data the *gsp* operon was mainly associated with strong biofilm formers. In planktonic cells, using NMP as the adjuvant, the MIC of ciprofloxacin, chloramphenicol, and tetracycline exhibited a two-, six- and ten-fold reduction, respectively, in comparison to the antibiotic alone. In the case of sessile cells, half showed reductions in biofilm biomass when tetracycline was combined with NMP. TEM imaging demonstrated cell wall damage with NMP.

Significance: Our findings showed that NMP damages the cell wall, increasing drug permeabilization. Use of NMP and NMP-like structures has the potential to reverse MDR in bacteria.

T5-09 Efficacy of Individual and Combinatory Antimicrobial Dip Treatments for the Control of *Listeria monocytogenes* on Fresh Cheese

SARAH KOZAK[1], Yustyna Bobak[2] and Dennis D'Amico[1]
[1]University of Connecticut, Department of Animal Science, Storrs, CT, [2]University of Connecticut, Department of Molecular and Cell Biology, Storrs, CT

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Introduction: Post-lethality contamination of fresh cheese with *Listeria monocytogenes* (Lm) has been the cause of several recent outbreaks of foodborne illness in the United States. With rapid growth during refrigerated storage, there is a critical need for strategies to control Lm on these products.

Purpose: The objectives of this study were to determine the efficacy of individual treatments of acidified calcium sulfate with lactic acid (ACSL; 25%), ε-polylysine (EPL; 10%), hydrogen peroxide (HP; 5%), lauric arginate (LAE; 2%, 5%), and sodium caprylate (SC; 10%) applied as aqueous dip solutions to control *Lm* on fresh cheese; and, to identify antimicrobial interactions of combinatory treatments of LAE+EPL, EPL+SC, ACSL+SC, and LAE+SC.

Methods: Samples of Queso Fresco (25 g), from three independent batches of cheese, were surface inoculated with an *Lm* cocktail to achieve ca. four log CFU/g. The cheese was submerged in antimicrobial solutions for 60 s, vacuum-packaged, and then stored at 7°C for 35 days. Counts of *Lm* were determined after 24 h and then weekly throughout storage.

Results: Overall, HP was the most effective bactericidal and bacteriostatic treatment, reducing counts to <one log CFU/g within 24 h and through 35 days. Treatment with SC was the only other single treatment with *Lm* counts significantly lower than the control count on day 35 (P<0.0001). The combination treatment of EPL+LAE initially produced a two log CFU/g inactivation, but over time there was no difference in *Lm* counts. In contrast, counts from the combination treatments of ACS+SC, EPL+SC, and LAE+SC were significantly lower than the control throughout storage and when each of the compounds was applied individually at day 35 (P<0.05). Though these combinations were effective, the only synergistic treatment identified was ACSL+SC.

Significance: These data indicate that HP and several combinatory dip treatments, notably ACSL+SC, are promising approaches for controlling *Lm* on fresh cheese throughout a 35-day shelf-life.

T5-10 Decontamination of Chicken Breast Meat, Romaine Lettuce Leaves, and Stainless Steel Surfaces from a Multidrug-resistant Strain of *Salmonella enterica* Serovar Heidelberg Using a 2D-Air-Based DBD-Plasma Microdischarge Array

HAMADA ABOUBAKR[1], Muhammad Nisar[1], Kakambi Nagaraja[1], James Collins[1], Peter Bruggeman[2] and Sagar Goyal[1]

[1]University of Minnesota, College of Veterinary Medicine, St. Paul, MN, [2]Department of Mechanical Engineering, University of Minnesota, Minneapolis, MN

❖ ***Developing Scientist Competitor***

Introduction: In the United States, 1.2 million illnesses are caused by *Salmonella* resulting in 19,000 hospitalizations and 380 deaths, annually. Poultry meat is the major implicated food, but contaminated fresh produce is also implicated. *Salmonella* Heidelberg (SH) is the most common serovar found in retail poultry. Antimicrobial drug resistance in this serovar is notable and has increased in recent years.

Purpose: Decontamination efficacy of a 2D-air-based DBD-plasma microdischarge array (2D-AMPA) against a multidrug resistant (MDR) SH isolate on stainless steel, romaine lettuce, and chicken breast was studied.

Methods: Samples (stainless steel discs, lettuce leaves, and pieces of chicken breast) were spiked with ~6.5 log CFU of MDR SH culture. After drying, the samples were exposed to the 2D-AMPA for one to 10 min at 10 cm distance and at optimal operational conditions. The surviving bacterial cells at each time were counted by plating serial 10-fold dilutions onto tryptic soy agar plates. The reduction in bacterial numbers was calculated by difference between bacterial counts with and without AMPA treatments.

Results: A complete reduction in bacterial counts (> six log CFU/ one cm² sample surface) was attained after four minutes exposure on stainless steel surfaces. However, complete reduction was not attained on lettuce and chicken breast even after 10 min of exposure. After five minutes exposure, ca. four and ca. three log reductions were seen on lettuce and chicken, respectively. A possible reason could be that the antibacterial efficacy of plasma decreases on organic and proteinaceous surfaces.

Significance: While plasma inactivation of *Salmonella* has been assessed previously, this was the first analysis of the efficacy of plasma inactivation of MDR SH on different foods and a food contact surface. The results showed that our cost-effective 2D-AMPA setup, which produces plasma at low power from air, is promising as an alternative technology for destroying MDR pathogens on poultry, fresh produce, and food contact surfaces.

T5-11 Use of Bacteriophage as an Additive during the Preparation of Ready-to-Eat Meat Products to Control *Listeria monocytogenes*

Hanie Ahmadi[1], Shai Barbut[1], Loong-Tak Lim[1] and **S. BALAMURUGAN**[2]

[1]University of Guelph, Guelph, ON, Canada, [2]Agriculture & Agri-Food Canada, Guelph, ON, Canada

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Introduction: Bacteriophages have been approved for use as a processing aid to be applied on the surface of RTE meat products prior to packaging. However, it is currently not clear if mixing bacteriophage within the meat, as an additive before thermal processing of RTE meats, would affect the anti-*Listeria* activity.

Purpose: The purpose of this study was to evaluate the efficacy of *Listeria* phage A511 when used as an additive before cooking, to RTE meat products, to control *L. monocytogenes*.

Methods: Model RTE thin meat products were prepared with common levels of sodium polyphosphate and salt, as well as phage at 10^8 PFU/g. Samples (10 g of meat slurry) were cooked to 71°C for 30 s, cooled, and then inoculated with 10^3 CFU *L. monocytogenes*/g . Samples were stored at 4°C and enumerated for bacteriophage and *L. monocytogenes* at predetermined time intervals, over a 28-day period, in triplicate.

Results: Phage numbers significantly (P<0.05) decreased during cooking by approximately 1.5 log PFU/g. Neither salt (2 and 3%) nor sodium polyphosphate (0.5 and 1%) had a significant (P>0.05) effect on phage stability. No significant difference (P>0.05) in *L. monocytogenes* numbers was observed between bacteriophage-treated samples and untreated controls, suggesting that bacteriophage used as an additive does not inhibit the growth of *L. monocytogenes.* This is probably due to immobilization of bacteriophages in meat matrix.

Significance: Results from this study suggest that the use of bacteriophage as an additive in RTE meat products before thermal processing is not effective in inhibiting the growth of *L. monocytogenes*.

T5-12 Evaluation of Recirculating Chlorinated Nanobubble Water to Control Shiga Toxin-producing *Escherichia coli* Surrogates in a Novel Commercial Ground Beef Production System

AMANDA WILDER[1], Nicholas Sevart[1], Anna Porto-Fett[2], John Luchansky[2], Harshavardhan Thippareddi[3], Christopher Vahl[4], Gary Acuff[5] and Randall Phebus[1]

[1]Kansas State University, Manhattan, KS, [2]U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, [3]University of Georgia, Athens, GA, [4]Kansas State University, Department of Statistics, Manhattan, KS, [5]Texas A&M University, College Station, TX

❖ ***Developing Scientist Competitor***

Introduction: A variety of antimicrobial processes are used to reduce pathogen risks on processed raw beef. Interest in nanobubbles as an antimicrobial has increased due to their proposed surfactant properties. No published research has evaluated nanobubble-infused chlorinated solutions generated using electrolyzed (EO) water on beef tissues.

Purpose: This study evaluates the effectiveness of recirculating, sanitizing chilled water infused with acidic EO water combined with nanobubble technology, in a proprietary raw beef manufacturing process, against a five-strain STEC surrogate cocktail, over a six-day commercial-scale processing scenario.

Methods: Inoculated beef trim (7.0 log CFU/g) was introduced into the chilled recirculating sanitizing water system [pH 5 and 5 ppm free available chlorine (FAC) targeted], twice daily every other day, over six days of continuous ground beef processing. Inoculated trim was sampled immediately following exposure to the sanitizing water (30 to 90 s). Uninoculated trim was sampled daily to determine residual surrogate contamination throughout the manufacturing system.

Results: Exposure to the sanitizing water reduced surrogate populations (P≤ 0.05) by 1.6 log CFU/g on inoculated meat. Approximately 2.7 log CFU/g of residual surrogates were recovered on uninoculated meat ~35 min after the inoculated meat entered the system; indicating that harborage of surrogates on equipment can subsequently contaminate product. Surrogates were recovered from uninoculated meat by enrichment only (0.4 log CFU/g detection limit) on alternate processing days, where no inoculated trimmings were introduced. Control of this carry-over surrogate contamination in the system, fol-

lowing inoculation, was accomplished through daily equipment sanitation and boosting FAC in recirculated processing water to 50 ppm during a four-hour system disinfection period at the end of each processing day.

Significance: Chilled, chlorinated nanobubble water was shown to be an antimicrobial process against surrogate bacteria representing enteric pathogens in this proprietary ground beef manufacturing system. Other applications of the antimicrobial technology may result based on these findings.

T6-01 Strategies for Enhanced Protection of Agricultural Produce in Outdoor Storage

CHIN GOUK, Simone Kreidl and Peta Faulkner

Department of Economic Development, Jobs, Transport and Resources, Melbourne, Australia

Introduction: Agricultural produce that are stockpiled in the field are generally covered with a waterproof tarp made of polyvinyl chloride (PVC) or polypropylene material to protect them from rain. A disadvantage of these materials is that moisture is trapped under the tarp and condensation drips back into the stockpile. The higher moisture content in turn promotes microbial growth. Tarps that are waterproof yet able to liberate moisture from agriculture produce are needed to reduce the risk of microbial contamination.

Purpose: The purpose of this study was to evaluate the performance of a breathable waterproof tarp (BWT) for protection of almond stockpiles.

Methods: The performance of a new and a reused piece of BWT was compared with a standard PVC tarp (STD) during the 2015 and 2016 seasons. Relative humidity (RH) and temperature at various depths in stockpiles were monitored using an array of smart sensors connected to a data logger. Tests were conducted on samples, in triplicate, collected at trial set up and six to nine weeks after storage to determine: (i) moisture content; (ii) microbial levels; and (iii) the incidence of spoilage.

Results: In comparison with almond stockpiles covered with an STD, those protected by the BWT had lower RH and less diurnal fluctuations. Consequently, almonds in the surface layers of stockpiles under the BWT, which were most at risk from condensation run-off, had significantly lower moisture contents; lower levels (36x) of spoilage fungi; lower levels (143x) of bacteria; and lower incidence (8x) of moldy kernels. The reused BWT was, also, able to limit microbial growth in almond stockpiles.

Significance: The BWT has ensured almonds remain dry during storage, with little increase in the level of spoilage. The BWT provided effective protection when reused for a second season, thereby improving its cost-effectiveness.

T6-02 The In Vitro and In Vivo Effects of *Pseudomonas aeruginosa Des*B on Pathogen-host Interaction

JIMYEONG HA[1], Sejeong Kim[1], Yohan Yoon[1] and Kyoung-Hee Choi[2]

[1]Sookmyung Women's University, Seoul, South Korea, [2]Wonkwang University, Iksan, South Korea

❖ ***Developing Scientist Competitor***

Introduction: Pseudomonas aeruginosa is an important food spoilage psychrotropic bacterium, which is widely found in environments such as soil, plants, and animal tissues. It produces many virulence factors that contribute to spoilage in foods and host defense/activation of inflammatory responses/host damage in human. Notably, this spoilage pathogen can cause harmful chronic lung damage in immunocompromised patients. Previous study showed that an aerobic desaturase (DesB) of P. aeruginosa exerted significant effect on virulence determinants.

Purpose: The objectives of this study was to analyze the in vitro and in vivo effect of DesB on host interaction and possibly to expand the comprehension of its pathogenic effect on food hosts, such as mice.

Methods: For the in vitro experiments, *P. aeruginosa* (WT) or a *desB* mutant was grown up. The bacterial cells and supernatants were collected by centrifugation and, then, diluted with F-12 supplemented with 10% FBS. The diluents were added to A549 cell monolayer for determining cell viability, invasion, and/or immune response. For the in vivo experiments, six to seven weeks old ICR mice were infected by endotracheal intubation with six to seven log CFU/ml bacterial cell for 24 h. After the mice were sacrificed, the survival rates of each strain, in the lungs, were measured. The histopathology of lung tissue was, also, observed.

Results: The *desB* mutant exhibited lower cytotoxicity to A549 cells than WT and stimulated secretion of a more pro-inflammatory cytokine (IL-6) and chemokines (GRO, IL-8, and MCP-1) compared to WT. In mouse model, the survival rate of the *desB* mutant was lower than for WT in the lungs.

Significance: The results suggested that *P. aeruginosa* DesB affects pathogenicity and microbe-host interactions.

T6-03 Occurrence of Sporeformers in Processed Milk from Household Refrigerators and the Effect of Heat Treatment on *Bacillus* Spore Activation

Sarisha Devnath[1], **AJIBOLA OYEDEJI**[1] and Oluwatosin Ademola Ijabadeniyi[2]

[1]Durban University of Technology, Durban, South Africa, [2]Durban University of Technology, Durban, South Africa

❖ ***Developing Scientist Competitor***

Introduction: Recently, milk contamination has become a major problem in households. This is largely due to the occurrence of sporeforming bacteria, even after the milk has been processed. One of such group of bacteria is *Bacillus*.

Purpose: This study investigated the occurrence of spore formers in processed milk samples collected from household refrigerators and the effect of pasteurization temperatures on *Bacillus* spores activation.

Methods: Twenty-four samples of ultra-high temperature (UHT) processed milk and pasteurized milk were collected from 24 households and analyzed for the presence of sporeformers. For the spore activation study, raw milk samples were collected from a local dairy farm and subjected to four different pasteurization regimes (65°C for 30 mins, 72°C for 15 s, 80°C for 30 mins, and 100°C for 2 s). Thereafter, pasteurized milk samples were stored for seven days at 5°C and 10°C and analyzed daily.

Results: The average number of aerobic sporeformers in UHT milk and pasteurized milk were 5.77 CFU/ml and 5.88 CFU/ml, respectively. DNA extraction and sequencing further revealed that the mixed culture contained *Bacillus cereus, Bacillus thuringiensis,* and *Bacillus toyonensis* for both pasteurized and UHT milk samples. The average number of aerobic sporeformers in raw milk for samples stored at 5°C ranged between 4.67 to 6.00 CFU/ml and 4.84 to 6.00 CFU/ml at 10°C. There was no significant increase in the number of colonies as storage days increased; however, significant decreases were observed ($P<0.05$) with increases in pasteurization temperature.

Significance: This work showed that household milk may constitute a public health risk. Also, pasteurization regimes that have been studied may not effectively inactivate all aerobic sporeformers in milk because of *Bacillus* spores activation.

T6-04 Thermal Death Kinetics of *Bacillus sporothermodurans* Spores Isolated from Ultra-high Temperature Milk

RODNEY OWUSU-DARKO, Lungile Shongwe and Elna Buys
University of Pretoria, Pretoria, South Africa

❖ ***Developing Scientist Competitor***

Introduction: *Bacillus sporothermodurans* is a rod shaped mesophilic bacterium producing highly heat resistant spores (HRS), which can survive ultra-high temperature (UHT) milk processing. The presence of these spores may have implications on the quality and safety of UHT milk and other thermally processed food products. Under UHT conditions, *B. sporothermodurans* spores have been found to be more resistant than other heat resistant spores, with *D*140 ranging from 3.4 to 7.9 s.

Purpose: The purpose of this study was to establish the heat inactivation kinetics of selected *B. sporothermodurans* strains with the aim of improving thermal validation during UHT food processing and to evaluate the performance of the linear, Weibull, and biphasic models of inactivation with the aim of establishing the best fit for *B. sporothermodurans* in UHT milk processing.

Methods: *Bacillus sporothermodurans* spores were prepared by dispensing one ml of culture onto the sporulation medium [Nutrient broth (25 g/liter), bacteriological agar (15 g/liter), vitamin B12 (1 mg/liter), $MnSO_4.H_2O$ (8.4 mg/liter) and $CaCl_2.2H_2O$ (1 g/liter), pH 6.8)] and then harvesting with physiological saline (8.5 g/liter). Sterile milk was inoculated with the bacterial spores to a concentration of approximately $2x10^7$spores/ml. Thermal inactivation analysis was undertaken and subsequent modeling of the data using the linear, Weibull and biphasic models.

Results: The survival curves indicated a good fit for the nonlinear models for the selected strains. At 130°C, tailing of curves started after approximately 30 s and 35 s treatment time, corresponding to a two-log reduction. The Weibull model consistently proved a better fit than the biphasic and linear models after computation of the mean square error (0.10, 0.14, and 0.77, respectively) and correlation coefficient (0.99, 0.98, and 0.86, respectively).

Significance: The Weibull model should provide the best model for use in thermal inactivation of *B. sporothermodurans* in UHT milk processing in the food industry.

T6-05 Desaturase-mediated Adaptation to High Salt Concentration in *Pseudomonas aeruginosa*

SEJEONG KIM[1], Jimyeong Ha[1], Yohan Yoon[1] and Kyoung-Hee Choi[2]
[1]Sookmyung Women's University, Seoul, South Korea, [2]Wonkwang University, Iksan, South Korea

❖ ***Developing Scientist Competitor***

Introduction: Bacteria have adaptation systems against environmental stresses, maintaining appropriate cell membrane fluidity for normal bacterial growth by adjusting the ratio of saturated fatty acid (SFA) and unsaturated fatty acid (UFA). One of the food spoilage bacteria, Pseudomonas aeruginosa, is usually exposed to hostile stresses such as salt in foods. In previous study, adaptability to salt in Saccharomyces cerevisiae increased by the introduction of Arabidopsis fatty acid desaturase.

Purpose: This study investigated the role of an aerobic desaturase of *P. aeruginosa* (DesB) on salt resistance.

Methods: Pseudomonas aeruginosa PAO1 (wild type; WT) and its derived mutants (harboring a mutation in UFA synthesis genes, such as desB, desT, desA, or fabA) were used in this study. After the bacterial cultures were exposed to osmotic stress, the growth of each mutant was compared. Transcriptional levels of WT and desB mutants through qRT-PCR analysis were compared to assess the molecular mechanism of DesB on the salt stress response. The role of DesB on salinity adaptation was phenotypically confirmed as follows: Betaine was added to medium as a complement of the osmoprotectant (NAGGN) leakage and the bacterial growth was compared.

Results: The growth of all strains was inhibited under the exposure to 0.5M or 1.0M NaCl. The desB mutant displayed more impaired growth compared to WT and other mutants, suggesting the function of DesB as a player in salt stress. Comparative transcriptional analysis showed that genes involved in the synthesis of osmoprotectants (trehalose, NAGGN, and hydrophilin) were highly expressed in WT in response to high salt, whereas rarely expressed in the desB mutant. Further, a decrease in osmoprotectant of the desB mutant was partially complemented by the addition of betaine.

Significance: The results of this study indicated that *P. aeruginosa* DesB played a role in adaptability to high salinity by positively regulating the synthesis of osmoprotectants.

T6-06 Can the Adsorption-Desorption State Affect *Salmonella* Thermal Inactivation Kinetics in Low-moisture Foods?

FRANCISCO GARCÉS-VEGA and Bradley Marks
Michigan State University, East Lansing, MI

❖ ***Developing Scientist Competitor***

Introduction: To comply with FSMA Preventive Controls Rules, processors of low moisture foods must establish and validate pathogen reduction steps. Validation can be accomplished in several ways, including predictive microbiology. However, predictive models must be valid for the product and process.

Purpose: The objective of this work was to evaluate the effect of adsorption-desorption hysteresis on *Salmonella* thermal inactivation on almonds.

Methods: Three batches of almonds (initially 0.36 aw) were inoculated (eight log CFU/g) with *Salmonella* Enteritidis PT30 and subjected to one of three adsorption-desorption treatments in controlled humidity chambers (10 to 30 days): (1) equilibrated by adsorption to 0.42 aw and 3.9% MC (moisture content, dry basis) , (2) equilibrated by adsorption to 0.62 aw and 5.9% MC, and then equilibrated by desorption back to 0.42 aw and 4.3% MC, or (3) equilibrated by adsorption to 0.46 aw and 4.2% MC. This yielded two samples at the same aw but different % MC (1 and 2), and two at the same % MC but different aw (2 and 3). Subsequently, samples were heated in a water bath (80°C, 1 h) and sampled every 10 min. Surviving *Salmonella* was enumerated on modified trypticase soy agar. The survival curves were analyzed using analysis of covariance.

Results: The inactivation rates were not significantly different ($P>0.05$). *D*-values ranged from 15-18 min, with a 0.3 min difference between treatments 1 and 2, a 2.3 min difference between treatments 1 and 3, and a 2.0 min difference between treatments 2 and 3.

Significance: Sorption and desorption state were not a significant factor in *Salmonella* thermal inactivation on almonds. There were similar trends for the *D*-values as a function of % MC and a_w. Further research is needed to test the effect of sorption state on other products with potentially larger hysteresis and to reduce the uncertainty of the inactivation parameters.

Technical

T6-07 Survival of *Salmonella* in Low-moisture Military Ration Products

Andre Senecal, **GENEVIEVE FLOCK**, Michelle Richardson, Courtney Cowell and Dominique Pacitto
U.S. Army NSRDEC, Natick, MA

Introduction: Survival of *Salmonella* in low-moisture foods (LMF) with low water activity (aw) have been implicated in increased foodborne outbreaks during the recent decade.

Purpose: This trend has prompted the Army to investigate the potential long-term survival of *Salmonella* in LMF, since they are frequently included in military rations and are required to have a minimum three year shelf-life at 25°C.

Methods: Simulated commercial products with low a_w, such as peanut butter (0.21), mocha dessert bars (0.43), boil-in-bag eggs (0.091), and chocolate protein drinks (0.34), were prepared with a dry inoculum of *Salmonella*. A five strain inoculum of *Salmonella* Agona, *Salmonella* Enteritidis, *Salmonella* Montevideo, *Salmonella* Tennessee and *Salmonella* Typhimurium was prepared and added to one ingredient in each product. The carrier ingredient was then added to a Robot Coupe to combine all product ingredients into the final ration item. Products were weighed into 40 g samples and vacuum sealed in tri-laminate pouches. Products were stored at 4°C, 25°C, or 40°C for up to three years. *Salmonella* was enumerated by dilution and plating on TSA and selective Hektoen Enteric Agar (HE) for each product at two weeks, one month, six months, one year, two years and three years.

Results: Results indicated no loss in *Salmonella* viability for peanut butter, chocolate protein drink, and boil-in-bag eggs when stored at 4°C and 25°C and an approximately three log loss when stored at 40°C for six months. Mocha desert bars, which had the highest a_w of the stored products, had a loss of approximately 0.5 log at 4°C, 1.5 log at 25°C, and a loss of seven log at 40°C after six months of storage.

Significance: The data from this study suggested that *Salmonella* can survive in low moisture military ration products at elevated temperatures for extended periods of time. This indicates the need for additional safety protocols.

T6-08 Heat Resistance of *Salmonella* spp. and *Enterococcus faecium* Increased Exponentially at Reduced Water Activity in Silicon Dioxide

SHUXIANG LIU, Ravi Kiran Tadapaneni, Meijun Zhu, Sablani Shyam and Juming Tang
Washington State University, Pullman, WA

❖ ***Developing Scientist Competitor***

Introduction: Thermal resistances of bacteria in low-moisture foods varies by strains, chemical/physical properties, and water activity (aw) of foods. The direct effects of aw on the kinetics of thermal inactivation of bacteria remains unclear.

Purpose: The objectives of this study were: (i) to evaluate silicon dioxide as a carrier to mimic a low moisture environment, and (ii) to assess thermal resistances of *Salmonella* Enteritidis PT 30 and its potential surrogate *Enterococcus faecium* NRRL B-2354 over a range of a_w at 80°C ($a_{w,80°C}$).

Methods: The equilibrium isotherms (moisture content versus water activity) of silicon dioxide at 80°C were obtained using a newly designed cell. Bacterial inoculums were inoculated to silicon dioxide and dried in the ambient environment. Samples (~ one gram) were sealed in custom designed test cells, heated isothermally (80°C) at the selected $a_{w,80°C}$, cooled, and plated on differential media. The $a_{w,80°C}$ (0.1 to 1.0) of inoculated samples was controlled in these cells by balancing water vapor pressure via solutions from 0 to 18mol/kg. The survivors were enumerated and used for $D_{80°C}$ calculation.

Results: Flat equilibrium isotherm curves of silicon dioxide were observed (a_w remained at 0.30±0.02). *Enterococcus faecium* showed higher $D_{80°C}$ values than those of *Salmonella* Enteritidis at all tested $a_{w,80°C}$. The $D_{80°C}$ for both bacteria increased exponentially when $a_{w,80°C}$ dropped from 0.7 to 0.1 (R^2=0.94 to 0.98, $D_{80°C}$ of *E. faecium/Salmonella* at $a_{w,80°C}$ 0.7 and 0.1 were 4.4±0.2/2.1±0.2 and 281.8±10.0/171.0±18.5 min, respectively).

Significance: Silicon dioxide can serve as a carrier for evaluating bacterial thermal resistance in low moisture environment. *Enterococcus faecium* appears to be a valid *Salmonella* surrogate at 80°C regardless of the $a_{w,80°C}$. The relationship between $D_{80°C}$ of both microorganisms in silicon dioxide and $a_{w,\ 80°C}$ can promote understanding of thermal resistances of bacteria in low-moisture foods.

T6-09 Evaluation of Survival on Flaxseeds and Subsequent Heat Resistance among Four *Salmonella* Serovars

SAHAR MALEKMOHAMMADI and Teresa Bergholz
North Dakota State University, Fargo, ND

❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* found in low-moisture foods has been limited to a few strains.

Purpose: Many *Salmonella* serovars have been isolated from low-moisture foods and have also been linked to outbreaks; however, the thermal resistance among serovars has not been thoroughly evaluated.

Methods: Since variation in survival and subsequent thermal resistance among *Salmonella* serotypes is unknown, we quantified survival over four months and subsequently determined the thermal resistance of 32 strains of *Salmonella* representing four different serotypes inoculated at eight log CFU/g onto flax seed.

Results: Reduction in cell numbers over time was nonlinear and survival/inactivation rate parameters were determined from the Geeraerd model. Over a four month period, the average kmax for *Salmonella* Enteriditis strains was 0.8±0.1; significantly higher than that for *Salmonella* Agona, which was 0.5±0.1. To quantify the subsequent heat resistance of *Salmonella* strains after two and four months of storage, inoculated flaxseeds were subjected to vacuum steam pasteurization at 72±1°C for 0.5, 0.75, 1, 1.25, 1.5, 2, and 3 min. After two months of storage, kmax values ranged from four to six. After four months of storage, average kmax was 7.0±2.0, 5.8±0.8, 4.6±1.2, and 4.3±0.8 for serotypes *Salmonella* Enteriditis, *Salmonella* Montevideo, *Salmonella* Tennessee, and *Salmonella* Agona, respectively. After four months, *Salmonella* Enteriditis had a higher inactivation rate than the other serovars. With increased storage time, the heat resistance of serovar Tennessee strains increased 2.3-fold.

Significance: These results showed that survival on flax seed varies among these four serotypes, with strains of serotype Agona exhibiting the highest survival after four months. Also, results demonstrated that adaptation to low moisture conditions increased the thermal resistance of serovar Tennessee.

T6-10 A Novel Method to Determine Thermal Death Kinetics of Microorganisms in Low-moisture Foods: Thermal-Death-Time Sandwich

SOON KIAT LAU[1], Harshavardhan Thippareddi[2] and Jeyamkondan Subbiah[1]
[1]*University of Nebraska-Lincoln, Lincoln, NE,* [2]*University of Georgia, Athens, GA*

❖ ***Developing Scientist Competitor***

Introduction: Traditionally, the thermal death kinetics of microorganisms in foods are determined using tubes, disks, pouches, or similar vessels in a water or oil bath. However, this method suffers from prolonged come-up time (CUT) for low moisture foods. A long CUT results in microbial destruction during the CUT which confounds the results. A heating device with fast-responding controls can reduce the CUT, thus increasing the precision of the thermal destruction parameters.

Purpose: This study was undertaken to develop a precision controlled heating system that reduces CUT and, subsequently, compare it to traditional thermal-death-time (TDT) disks immersed in an oil bath.

Methods: A TDT sandwich consists of a sample packaged in an aluminum-lined pouch that is sandwiched between resistive heating pads adhered to aluminum plates. The TDT sandwich was insulated with ceramic fiber boards and controlled with an Arduino microcontroller. Pouches filled with 10.0 g of water were heated to 90°C for 15 min to test the permeability of the pouches. The CUT (time taken to reach 90±0.5°C) of the TDT sandwich was compared against traditional oil bath method using 0.5 g samples of ground black pepper. Samples for the oil bath were packaged in aluminum TDT disks. All samples were tested in at least triplicate.

Results: The moisture loss in water samples after heating was 0.00027±0.00016 g, indicating low moisture permeability of the pouch material at 90°C. The ground black pepper CUT to attain 90°C was 26.3±1.7 s using the TDT sandwich system, whereas the CUT in the oil bath was between 190.2±26.6 s and 283.2±35.1 s, depending on the location.

Significance: The TDT sandwich method had shorter and more consistent CUTs compared to the traditional oil bath method. The low moisture permeability and dry nature of the pouches also avoided leakage or contamination of samples during thermal treatment.

T6-11 Microbial Safety of Edible Low-water Activity Foods: Study of Simulated and Durban Household Samples

OLUWATOSIN ADEMOLA IJABADENIYI[1] and Yovani Pillay[2]
[1]*Durban University of Technology, Durban, South Africa,* [2]*Durban University of Technology, Durban, South Africa*

Introduction: Low water activity (aw) foods have been increasingly linked with foodborne illness outbreaks and product recalls, which are mainly due to isolation of foodborne pathogens.

Purpose: This study was undertaken to determine the presence and survival of pathogens of importance in selected low a_w foods.

Methods: Sixty household low a_w foods were examined for the presence of selected pathogens using conventional methods and a simulative study was conducted using a high sugar, low a_w macadamia butter to determine the survival of *Bacillus cereus* and *Staphylococcus aureus* ATCC 25923.

Results: Results obtained from 60 low a_w household samples had some significant differences ($P \leq 0.05$) within food categories. There were no significant differences ($P \geq 0.05$) amongst the different food groups; except for spices and nuts, which had significant differences in their colony counts compared to those of other food categories. Spices had the highest number of aerobic bacteria, aerobic sporeformers, anaerobic sporeformers and *S. aureus*. Mean aerobic colony counts for nuts and spices were 2.30 log CFU/g and 4.40 log CFU/g, respectively. Pathogens such as *Escherichia coli* and *Cronobacter sakazakii* were present in nuts, whilst *Salmonella* spp. were present in chocolates. In the simulative study, temperature and high sucrose concentrations played a significant role in the survival of *B. cereus* and *S. aureus*. *Bacillus cereus* was found to be more osmotolerant at both reduced and elevated temperatures (18 to 25°C) in the butter samples with 12% sucrose; whilst, *S. aureus* seemed to grow better in sucrose free samples at both temperatures. After 28 days of storage of 20% sucrose sample, *S. aureus* was not detected and there was four-log reduction of *B. cereus*.

Significance: This work implied that certain low a_w foods may present a public health risk. The simulated work also signified that *B. cereus*, being a spore forming bacterium, can be osmotolerant at both reduced and elevated temperatures.

T6-12 Utilization of *Enterococcus faecium* as a *Salmonella* spp. Surrogate for Thermal Treatment in Selected Low-moisture Food Products

NURUL AHMAD[1], Ian Hildebrandt[1], Shannon Pickens[2], Soon Kiat Lau[3], Jie Xu[4], Shuxiang Liu[4], Hsieh-Chin Tsai[4], Angela Maria Rincon[5], Jeyamkondan Subbiah[3], Harshavardhan Thippareddi[5], Meijun Zhu[4], Juming Tang[4], Nathan Anderson[6], Elizabeth Grasso-Kelley[2], Elliot Ryser[1] and Bradley Marks[1]
[1]*Michigan State University, East Lansing, MI,* [2]*Illinois Institute of Technology, Bedford Park, IL,* [3]*University of Nebraska-Lincoln, Lincoln, NE,* [4]*Washington State University, Pullman, WA,* [5]*University of Georgia, Athens, GA,* [6]*U.S. Food and Drug Administration, Bedford Park, IL*

❖ ***Developing Scientist Competitor***

Introduction: Composition and structure of low moisture foods may affect *Salmonella* thermal resistance. Therefore, it is important to qualify the appropriateness of surrogates prior to use in validating preventive controls.

Purpose: This study aimed to compare thermal resistance of *Enterococcus faecium* and *Salmonella* in peanut butter, almond meal, wheat flour, nonfat dried milk powder, date paste, and ground black pepper; and to determine reproducibility of results across five laboratories.

Methods: Each of six low-moisture foods were assigned to two laboratories, with the lead laboratory inoculating samples, testing homogeneity, and equilibrating to the desired water activity (aw) prior to shipping. The products were inoculated with *E. faecium* NRRL B-2354 or a five-strain *Salmonella* cocktail (Agona, Typhimurium, Montevideo, Mbandaka, Enteritidis - all from low-moisture products). Both laboratories subjected samples to three isothermal heat treatments and survivors were enumerated on differential media.

Results: Overall, *Salmonella* showed lower ($P<0.05$) thermal resistance than *E. faecium* in almond meal, peanut butter, and nonfat dried milk powder. Although almond meal and peanut butter have a similar fat content, *E. faecium* and *Salmonella* showed less thermal resistance in almond meal (*D90°C Ef*: 8.55±0.13 min; *D90°C Sal* : 7.24±0.08 min at 0.45 aw) than in peanut butter (*D90°C Ef*: 21.17±0.21; *D90°C Sal*: 11.45 ± 0.25 min at 0.25 aw). Milk powder, at 0.25 aw , showed greater thermal resistance of *E. faecium* (D*90°C*: 25.20 ± 0.94 min) and less thermal resistance for *Salmonella* (*D90°C*: 6.69±0.13 min at aw0.25) than in almond meal and peanut butter.

Significance: Overall, *E. faecium* appears to be a robust surrogate for *Salmonella* spp. across multiple low moisture products (with multiple cross-laboratory validations); however, product characteristics significantly impact that relationship. It is, therefore, necessary to consider product composition when validating thermal processes for low aw foods.

T7-01 Cantaloupe Fruit Microbiome: Responses to Field Location, Cover Crop, and Cold Storage

Maria Albarracin[1], Christopher Gunter[1], Penelope Perkins-Veazie[2], Benjamin Chapman[1], Jonathan Baros[2], Sophia Kathariou[1] and **EDUARDO GUTIERREZ-RODRIGUEZ**[1]

[1]North Carolina State University, Raleigh, NC, [2]North Carolina State University, Kannapolis, NC

Introduction: Seasonal rain events during fruit formation and close to harvest are common during East Coast cantaloupe production and can result in soil/debris coming into contact with the fruit surface. Utilization of row cover crops, as physical barriers between soil and fruit, could be an alternative practice to prevent fruit contamination.

Purpose: This study was a determination of the effect of cover crops during cantaloupe cultivation in reducing soil/plant residues and pathogen contamination during the growing season.

Methods: Cantaloupe was grown at three different locations following commercial practices. At each location, three different row cover crops (Buckwheat (*Fagopyrum esculentum*), sunhemp (*Crotolaria juncea*), and mustard (*Brassica sp.*)) were grown between rows forming a physical barrier between soil and fruit. Cantaloupe was hand harvested, kept in individual bags according to field location and cover crop practice and stored for 24 to 36 h at 15°C before processing. Aerobic plate counts (APC) and presence of generic *Escherichia coli,* coliforms, *Enterococci* spp., *Salmonella* spp., Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* were determined from fruit surfaces after harvest and storage at 4°C (two weeks).

Results: Soil and cantaloupe microbial communities varied only by location. *Salmonella* spp. was present at 7, 60, and 0% in soil collected from locations 1, 2 and 3, respectively. *Listeria monocytogenes* was not detected; neither on soil nor fruit samples. STEC was present in soil from all locations at 60, 13, and 10%, respectively. Cantaloupe APC, *Enterococci spp.*, coliforms, and generic *E. coli* populations were significantly higher (P<0.05) in location 1 than 2 or 3. Irrespective of location and cover crop, cantaloupe APC*, Enteroccocci* spp., coliforms, *Salmonella* spp., and STEC significantly decreased during cold storage (4°C). *Salmonella* spp. and STEC persisted for up to two weeks during cold storage on fruit harvested from locations 1 and 2 (18 and 12%, respectively).

Significance: Preharvest pathogen cross-contamination of cantaloupe were mainly impacted by agronomic practices within each location, irrespective of mitigation practice.

T7-02 Fate of Shiga-toxigenic *Escherichia coli* in Central Florida Surface Waters at Different Temperatures

ZEYNAL TOPALCENGIZ[1] and Michelle D. Danyluk[2]

[1]Muş Alparslan University, Muş, Turkey, [2]University of Florida, Lake Alfred, FL

Introduction: Agricultural water can be contaminated directly by humans, animal activities, agricultural inputs, and environmental sources. Pathogens may be able to survive in agricultural water for significant periods of time.

Purpose: The objective of this study is to determine the fate of Shiga toxigenic *Escherichia coli* (STEC) and generic *Escherichia coli* in Central Florida agricultural surface water at different temperatures.

Methods: Five rifampicin (Rif) resistant STEC strains (O145, O104, O111, O103, O157) strains and naladixic acid (Nal) resistant generic *E. coli* (K-12) were prepared as one cocktail and inoculated into 100 ml agricultural water samples from two ponds to a final concentration of 10^5 CFU/ml. The same inoculation procedure was applied to sterilized surface water and EPA worst case water. Duplicate experiments with triplicate samples were performed at 15 and 25±1°C (n=6). *Escherichia coli* was enumerated by spread plating onto tryptic soy agar supplemented with Rif and Nal or a three-by-four tube MPN method (when no colonies were observed) for up to 168 days. The effect of protozoans was tested in a 14-day experiment in non-sterile agricultural surface waters with cycloheximide at all temperatures (n=3).

Results: STEC and generic *E. coli* concentrations decreased 5.4 log CFU/ml, on average, and reached ≤0.5 log CFU/ml in nonsterile surface water at all tested temperatures by day 168 (P>0.05); populations were significantly (P≤0.05) higher in sterile surface water and EPA worst case water at all sampling points beginning at day 28. Addition of cycloheximide to reduce protozoa grazing had no significant effect on behavior of *E. coli* populations.

Significance: Monitoring changes in the generic *E. coli* (represented by K-12) population is a reasonable indicator of STEC survival in agricultural water. Population reductions of *E. coli* in nonsterile agricultural water were not primarily driven by protozoa grazing, but rather by factors not evaluated here.

T7-03 Spatiotemporal Variability in Microbial Quality of Agricultural Water Supplies: Implications for Cooperative Sampling

MELISSA L PARTYKA, Ronald F. Bond, Jennifer A. Chase and Edward R. Atwill

University of California-Davis, Davis, CA

❖ ***Developing Scientist Competitor***

Introduction: The Produce Safety Rule (PSR) requires growers of covered commodities to inspect, evaluate, and monitor the quality of irrigation water through routine testing for generic *Escherichia coli*. While each grower is responsible for documenting their own water quality profile (WQP), the law also allows growers to utilize alternative sampling, i.e. developing a joint WQP from common sources, provided there are no potential sources of variability between the sampling site and point of delivery.

Purpose: This study was conducted to (i) evaluate potential drivers of bacterial variability in surface water sources and, (ii) examine the spatial scale at which samples maintained statistical similarity in order to facilitate cooperative sampling strategies.

Methods: Indicator bacteria (*E. coli* and fecal coliforms) were enumerated in water samples collected from 66 sites across six irrigation districts in central Washington and northern California on a monthly basis through the 2015 irrigation season (n=517). Prevalence of pathogens (*Salmonella* spp., *E. coli* O157 and shiga toxin-producing *E. coli* (STEC)) was determined for all one liter samples and subset (n=149) of high-volume (10 liter) samples. Physiochemical water parameters, in situ meteorological data, and a detailed environmental assessment were also included in the geospatial and statistical analyses.

Results: *Escherichia coli* results exceeding the PSR standard of 126 CFU/100 ml were rare (55 of 517) and were not associated with pathogen occurrence in 1 or 10 liter samples (P>0.05). *Escherichia coli* exhibited strong seasonal (P<0.05) and regional trends (P<0.001) with highest concentrations in California occurring during the late Spring. Significant spatial autocorrelation (P<0.01) was evident at the 5-10 km-scale and was not explained by environmental characteristics (P>0.05), including adjacent land uses.

Significance: This preliminary study suggested that adjacent sampling sites share similar water quality characteristics, which could facilitate cooperative sampling amongst growers sharing common irrigation sources. Additional sampling at finer spatiotemporal scales is ongoing and necessary to resolve site-specific trends.

T7-04 Prevalence of *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes* in Non-traditional Irrigation Waters in the Mid-Atlantic United States: A CONSERVE Project

ERIC HANDY[1], Cheryl East[1], Mary Theresa Callahan[2], Sarah Allard[3], Hillary Craddock[4], Shirley Micallef[2], Kalmia Kniel[5], Fawzy Hashem[6], Salina Parveen[6], Eric May[6], Joseph Haymaker[6], Amy Sapkota[3] and Manan Sharma[1]

[1]U.S. Department of Agriculture–ARS, Beltsville, MD, [2]University of Maryland, College Park, MD, [3]Maryland Institute for Applied Environmental Health, University of Maryland, School of Public Health, College Park, MD, [4]Maryland Institute for Applied Environmental Health, University of Maryland, College Park, MD, [5]University of Delaware, Newark, DE, [6]University of Maryland Eastern Shore, Princess Anne, MD

Introduction: Surface and nontraditional irrigation water (SNIW) sources can increase the irrigation water supplies without consuming potable water. However, these sources must be evaluated for enteric pathogens that could adulterate crops intended for human consumption and comply with FSMA irrigation water standards.

Purpose: This study evaluated surface (nontidal freshwater (NF), tidal freshwater (TF), pondwater (PW), tidal brackish (TB)) water sources from eight sites in Maryland for the presence of *Salmonella* spp., *Listeria monocytogenes (Lm)*, and *Escherichia coli* (*Ec*).

Methods: SNIW from eight sites (four NF, one TB, one TF, and two PW) were collected by traditional methods or filtered through modified Moore swabs. Sampling occurred on six different dates (September to December, 2016). Filters were quantified for *E. coli* populations (n=52) through standard methods (EPA 1604); Moore swabs were quantified for *Salmonella* spp. (n=32); and *Lm* populations (n=36) through a modified MPN procedure using 10-liter, 1-liter, and 0.1-liter volumes. Chemical parameters of water were measured. Culture-positive results for *Salmonella* spp. and *Lm* were confirmed through real-time PCR. One-way ANOVA on recovered pathogen populations in JMP were performed.

Results: Mean *Salmonella* spp. populations were 0.15+0.30, 0.11+0.10, 0.02+0.14, and 0.007+0.012 MPN/100 ml from NF, TF, TB, and PW sources, respectively. Mean *Lm* populations were 0.20+0.08, 0.28+0.17, 0.004+0.15, and 0.003+0.10 MPN/100 ml, respectively. Mean *Salmonella* spp. populations were significantly (P<0.05) higher at one NF site (MD05) compared to other sites. *Lm* populations at one NF site (MD08) were, also, significantly higher compared to other sites. From all water samples, 65% and 46% were positive for *Salmonella* spp. and *Lm*, respectively, and 53% contained *Ec* populations >126 CFU/100 ml. There were no significant correlations observed between *Ec* and either *Lm* or *Salmonella* spp. populations.

Significance: These data indicated that *Salmonella* spp. and *Lm* were present in multiple SNIW sources in Maryland. Year-round sampling is required to truly assess prevalence in this region.

T7-05 Evaluation of Cover Cropping, Farming System, and Meteorological Factors on the Survival of Generic *Escherichia coli* and *Listeria innocua* in Produce Fields

HAO PANG, Shirley Micallef, Kathryne Everts and Abani Pradhan

University of Maryland, College Park, MD

❖ ***Developing Scientist Competitor***

Introduction: Since soil can serve as a reservoir and contamination route for foodborne pathogens in produce production environments, it is necessary to evaluate the effect of various factors on the survival of foodborne pathogens in produce fields.

Purpose: This study sought to investigate the effect of a particular farm practice, cover cropping, along with meteorological factors on the survival of generic *Escherichia coli* and *Listeria innocua* in organic and transitional organic produce fields.

Methods: Five cover crop and one bare ground (no cover crop) control plots were inoculated with indicator bacteria generic *E. coli* and *L. innocua* in fall 2013 and 2014. Soil samples were collected periodically and were enumerated for *E. coli* and *L. innocua* by a modified MPN method. Survival analysis and Poisson regression were applied to determine the effects of cover crop, farming system, and meteorological factors on the survival of *E. coli* and *L. innocua* in soil.

Results: Survival analysis indicated that cover crop treatment was not a significant factor affecting survival of *E. coli* in soil. Interestingly, Cox regression revealed that survival of *E. coli* in soil was significantly associated with precipitation (P<0.001) and farming system (P=0.007). Increasing precipitation increased the survival of *E. coli;* and, the survival of *E. coli* in organic plots was longer than in transitional organic plots. For *L. innocua*, population levels were significantly higher in transitional organic plots as compared to organic plots. Significantly higher population levels were also observed under higher monthly precipitation, relative humidity, and temperature.

Significance: Our analyses revealed that while the effect of cover cropping is minimal, survival of food safety indicator bacteria are influenced by farming system. Moreover, increasing precipitation and humidity may prolong the survival of *E. coli* and persistence of *L. innocua* in soil in regions with cold weather.

T7-06 Persistence and Transmission of *Escherichia coli* and *Salmonella* spp. in a Watermelon Field Ammended with Poultry Litter: Year Two

Thais De Melo Ramos[1], Shani Craighead[1], Patrick Spanninger[1], Claire Marik[1], Samantha Gartley[1], Adam Vanore[1], Gordon Johnson[1], Manan Sharma[2] and **KALMIA KNIEL**[1]

[1]University of Delaware, Newark, DE, [2]U.S. Department of Agriculture–ARS, Beltsville, MD

Introduction: Poultry litter (PL) is a nutrient-rich soil amendment for crop production. However, the use of PL in soils may introduce enteric pathogens to fruits and vegetables.

Purpose: This study evaluated the persistence of a nonpathogenic, rifampicin-resistant *Escherichia coli* strain (TVS 355) and the presence of naturally occurring *Salmonella* spp. in soil, soil amended with PL, and on field-grown watermelons.

Methods: From May to October, 2016, twelve individual plots were seeded with Exclamation and Liberty watermelons planted on plastic mulch. Plots were unamended, unamended and inoculated with *E. coli*, amended with PL, or amended with *E. coli* inoculated PL. Soil samples were collected weekly (n=348). Watermelons (n=108) were swabbed at harvest. Samples were analyzed for *E. coli* populations by direct plating or MPN, and for *Salmonella* spp. by MPN using RV and TT broth or by enrichment using a modified FDA BAM procedure.

Results: By day eight, *E. coli* levels were significantly (P<0.0001) greater in PL-amended soils (5.02 log CFU/ gdw (gram dry weight) compared to unamended soils (3.98 log CFU/gdw). By day 120, *E. coli* populations in PL-amended soils were significantly (P<0.0001) greater (1.15±0.66 log CFU/gdw) compared to those in unamended plots which were below (0.12±0.01 log CFU/gdw). *Salmonella* spp. were detected in 62% of soil samples. By day eight the mean *Salmonella* spp. populations recovered from PL-amended and unamended plots were 1.36±0.40 log MPN/gdw and 1.08±0.27 log MPN/gdw, respectively. After 120 days, *Salmonella* spp. counts were 1.21±0.57 log MPN/gdw from PL-amended and 1.69±0.72 log MPN/gdw from unamended plots. *Salmonella* spp. were detected on watermelons (28 of 108) grown in both amended and unamended soils, containing *E. coli* populations that ranged from 1.95 to 2.81 log MPN/gdw. Approximately 92% of watermelons swabbed were positive for *E.coli*.

Significance: PL-amended soils provided conducive environments for *E. coli* persistence throughout the growing season and facilitated transfer to watermelons.

T7-07 Association of Fresh Produce Food Safety Hazard with Growth and Persistence of *Escherichia coli* in Soils Amended with FSMA-compliant Heat-treated Manure

PATRICIA MILLNER[1], Kathryn White[1], Herbert Clark[2], Fawzy Hashem[2] and Manan Sharma[1]
[1]U.S. Department of Agriculture–ARS, Beltsville, MD, [2]University of Maryland Eastern Shore, Princess Anne, MD

Introduction: Heat-treated, manure-based amendments are used for soil conditioning/fertilizing organically and conventionally grown fresh leafy greens and root crops. The risk of contamination from growth, persistence, and ultimately transfer of fugitive fecal bacterial pathogens to fresh produce upon contact between these preplant and side-dress amendments and crops is uncertain. Determining the impacts from use of FSMA-compliant soil amendments to grow produce is critical for devising science/risk-based food safety practices.

Purpose: This study investigated associations between heat-treated manure-based soil amendments and growth, survival, and transfer of *Escherichia coli* to spinach and radish.

Methods: Two randomized complete block field experiments (n=4 replications each) with soil amendments [composted poultry litter, composted dairy manure, vermicomposted dairy manure, heat-treated poultry litter pellets (PLP), and urea (control)] were conducted. Plots, spray-inoculated with a three-strain cocktail of rifampicin-resistant generic *E. coli* isolates (gEc^r), were tilled, seeded, overhead irrigated, and assayed 14 times (0 to 91 days) for gEc^r. Spinach leaves and radish were assayed (28 to 84 days) for gEc^r. Radish globes were washed, trimmed, sanitized (10 to 50 mg/L HOCl, pH6.5) for five minutes, then neutralized, and assayed for gEc^r.

Results: Populations of gEc^r in plots were ~ three log CFU/g soil (day 0) and increased 10- to 100-fold (day 1) for compost/urea and PLP treatments, respectively. The gEc^r population persisted 84$^+$ days in compost- and PLP-amended plots, but these organisms were barely detectable in urea treatments (day 28). Average gEc^r populations on radish from compost and PLP plots were 1.17 and 3.29 log MPN/globe, respectively, and significantly ($P<0.05$) greater than on spinach leaves. Serial washings removed visible soil from radish globes (n=72) and reduced, but did not completely eliminate, gEc^r from trimmed, chlorine-sanitized globes (n=48).

Significance: Heat-treated PLP, unlike composted manures or urea, promoted significant growth of gEcrin soil and transfer onto produce in direct contact with soil. Additional studies are needed to determine quality factors in commercially available heat-treated PLP responsible for this growth promotion.

T7-08 Differential Tissue Distribution of Internalized Human Norovirus, Porcine Sapovirus, and Tulane Virus in Lettuce and Spinach Plants

MALAK ESSEILI[1], Sarah Tegtmeier[1], Linda Saif[1], Tibor Farkas[2] and Qiuhong Wang[1]
[1]Ohio State University, Wooster, OH, [2]Louisiana State University, Baton Rouge, LA

Introduction: Lettuce has been implicated in human norovirus (HuNoV) outbreaks. We previously showed that HuNoV specifically binds to and persists on the surface of lettuce leaves; however, the dynamics of virus internalization into lettuce has not been fully investigated.

Purpose: This investigation assessed the internalization and tissue distribution of HuNoV and its surrogate viruses, porcine sapovirus (SaV) and Tulane virus (TV), in leafy greens.

Methods: Lettuce and spinach plants (n=6/time point/plant) were inoculated with HuNoV GII.4 (10^8 GE/ml), SaV and TV (10^6 $TCID_{50}$/ml) either through: (i) the seedlings' roots with viruses tracked on days one and six in the roots and leaves; or (ii) the cut petiole of mature leaves with viruses tracked on days one and three in the central veins, lower and upper leaf lamina. Processed tissues were subjected to RNase treatment prior to RNA extraction and virus-specific RT-qPCR. The infectivity of SaV and TV was determined in tissue culture.

Results: In both lettuce and spinach: (i) HuNoV translocated from roots to leaves at similar RNA titers; whereas, TV and SaV were retained at significantly higher RNA titers in the roots; and (ii) HuNoV disseminated with no tissue preference inside the leaves, while SaV and TV RNA titers were detected at significantly higher titers in central veins as compared with the upper and lower leaf lamina. Although TV and SaV were detected at comparable RNA levels, infectious SaV was detectable only in roots and veins. Infectious TV was detectable in all tissues, suggesting a higher stability of TV compared with SaV.

Significance: HuNoV internalized through roots and cut leaves and disseminated similarly into various spinach and lettuce tissues, raising concerns of internal contamination through irrigation and/or wash water. The internalization patterns of SaV and TV did not mimic HuNoV; this limits their utility as surrogate viruses.

T7-09 Risk Assessment of Factors Associated with the Occurrence of *Escherichia coli* O157:H7 on Cow/Calf Operations in Oklahoma and Louisiana

JOYJIT SAHA, Buddhini Jayasundera, Ravirajsinh Jadeja and Divya Jaroni
Oklahoma State University, Stillwater, OK

❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* O157:H7 poses a significant health-care concern and is an economic burden. It is part of the commensal microflora of ruminant animals, particularly cattle, and is shed intermittently in feces. On-farm management practices could affect occurrence and transmission in beef cattle and related products. Recognizing risk factors associated with the management practices at cow/calf operations is important to implementing effective measures against E.coli O157:H7.

Purpose: This study used multilevel modeling to quantify farm-practice risks associated with the occurrence of *E.coli* O157:H7.

Methods: Fecal, water, sediments, and equipment swab samples were collected from cow/calf operations in Oklahoma and Louisiana, over a five-year period. Each farm was visited twice yearly. Collected samples were tested for *E. coli* O157:H7 using culture and molecular techniques. Farm practices were assessed using a questionnaire covering water source/container, cattle breed, herd density, type of feed, and farm-cleanliness (Likert scale of one to five). Associations between occurrence of pathogens and management practices was determined using a multivariable logistic regression model.

Results: In Oklahoma and Louisiana, *E.coli* O157:H7 was present in 1.8 and 11.7% fecal, 4.4 and 21% water, 7.2 and 46% sediments, and 1.7 and 15.2% equipment swab samples, respectively. The odds of detecting *E.coli* O157:H7 in feces was lower than in sediments, water troughs, and equipment swabs. This suggests horizontal transfer of pathogens between the studied variables. Modeling indicated that using surface water increased the odds-ratio (OR) of a farm being positive for *E. coli*O157:H7 by 3.12 times that of groundwater. The OR increased with herd density, but decreased on farms with a combination of pure- and crossbreeds compared to those with crossbreeds, only. The OR was lower for grain-feed than for hay/commercial feed. Among farm-cleanliness factors, OR increased for equipment (3.6), chute (1.1), and alleyway (1.5), when cleaned rarely compared to quite often.

Significance: Quantification of risks associated with farm practices may aid in developing efficient ways to manage pathogen occurrence and transmission.

T7-10 Multiplex PCR-based Identification of Shiga Toxin-producing *Escherichia coli* Other Than the Top Seven Serogroups Found in the Feces of Feedlot Cattle

JUSTIN LUDWIG, Xiaorong Shi, Lance Noll, Jianfa Bai and T G Nagaraja
Kansas State University, Manhattan, KS

Introduction: *Escherichia coli* serogroups O26, O45, O103, O111, O121, O145, and O157 (Top 7) are major STEC foodborne pathogens. Cattle are a reservoir for the Top 7 STEC, which reside in the hindgut and are shed in the feces. Cattle harbor a number of other STEC serogroups (*n*=108), but not all of them have been shown to be associated with human illness.

Purpose: Our objective was to develop and utilize several sets of multiplex PCR assays to identify STEC isolates from feedlot cattle feces that were negative for the Top 7 STEC.

Methods: A total of 359 *E. coli* strains, positive for *stx*1 (Shiga toxin 1; *n*=65), *stx*2 (Shiga toxin 2; *n*=306), *stx*1 and *stx*2 (*n*=16), and/or *eae* (intimin; *n*=68) genes and negative for the Top 7 STEC were used. One hundred eighteen isolates were from fecal samples collected from a single feedlot in 2013 and 241 strains ertr isolated from pen-floor fecal samples collected from eight feedlots in the central United States. Several sets of multiplex PCR assays targeting serogroup-specific genes were used to identify the serogroups.

Results: Of the 118 strains from the single feedlot, 84 (71.2%) belonged to seven serogroups and 34 (28.8%) were unidentifiable. The seven serogroups included: O168 (29.7%), O109 (20.3%), O171 (9.3%), O104 (4.2%), O2 (4.2%), O8 (2.5%), and O175 (0.8%). Among the 241 strains from eight feedlots, 164 strains (68.0%) belonged to 13 serogroups and 77 (32.0%) were unidentifiable. The 13 serogroups included O168 (29.9%), O109 (16.2%), O2 (8.3%), O104 (5.0%), O8 (2.9%), O171 (1.7%), O136 (1.2%), O178 (0.8%), O76 (0.4%), O113 (0.4%), O118 (0.4%), O175 (0.4%), and O98 (0.4%).

Significance: Cattle harbor a number of STEC serogroups other than the those included in the Top 7. This illustrates the diversity of STEC shed in the feces. The two most predominant serogroups identified were O168 (107 of 359: 29.8%) and O109 (63 of 359; 17.6%).

T7-11 *Escherichia coli* and *Salmonella* Derby Carry a Novel Family of Temperate Bacteriophages that Encode Extended-spectrum Beta-lactamase Genes

ANNA COLAVECCHIO[1], Michca Gordon[2], Julie Jeukens[3], Jean-Guillaume Emond Rheault[3], Luca Freschi[3], Irena Kukavica-Ibrulj[3], Roger Levesque[3] and Lawrence Goodridge[1]
[1]McGill University, Ste-Anne-de-Bellevue, QC, Canada, [2]McGill University, St-Anne De Bellevue, QC, Canada, [3]IBIS, University of Laval, Quebec, QC, Canada

❖ ***Developing Scientist Competitor***

Introduction: Temperate bacteriophages (phages) constitute a source of genetic diversity for their hosts by encoding virulence factors and antibiotic resistance genes.

Purpose: The objective of this study was to phenotypically and genomically characterize three novel, extrachromosomal, temperate phage-like plasmids designated AnCo1, AnCo2, and AnCo3.

Methods: Whole genome sequencing was performed on two antimicrobial resistant *Escherichia coli* isolated from bovine feedlot associated wildlife and a clinical isolate of *Salmonella* Derby. Bioinformatic analysis was performed using RAST, PHASTER, ResFinder, and MEGA7 for annotation, identification of prophage regions, identification of antibiotic resistance genes, and construction of a phylogenetic tree, respectively. The phage-like plasmids were induced by mitomycin-C and confirmed by PCR, targeting the integrase gene. Electron microscopy was conducted for morphological characterization.

Results: AnCo1 and AnCo2 from wildlife *E. coli* isolates were determined to be 112,201 bp and 109,071 bp, respectively, and carried the extended-spectrum β-Lactamase CTX-M-15 gene, which was flanked by the mobile genetic element, ISE*cp1*. AnCo3 from the *Salmonella* Derby isolate was 105,994 bp with homology to phage-like plasmid pSTM_phi, and did not encode any antibiotic resistance genes. Plasmid extraction, PCR and genomic analysis suggested that the chromosomes of AnCo1 and AnCo2 were circular and extrachromosomal and had homology to *E. coli* phage-like plasmid pECOH89. AnCo1 and AnCo2 were inducible with mitomycin-C and electron microscopy demonstrated that AnCo1 had a large head (110 nm in diameter) and a long noncontractile tail characteristic of the *Siphoviridae* family. A maximum-likelihood tree constructed using the integrase gene as a marker for temperate phage diversity revealed that AnCo1, AnCo2, AnCo3, and seven published phage-like plasmids were located within their own branch. This suggested that they represent a novel family of phages.

Significance: AnCo1, AnCo2, and AnCo3 are novel phage-like plasmids that could potentially spread antibiotic resistance determinants among foodborne bacteria along the farm-to-fork continuum.

T7-12 Mathematical Modeling Approach for Enhancing Preharvest Sampling Plans for the Detection of Pathogenic Bacteria through Consideration of Prior Knowledge of Factors Related to Nonrandom Contamination

AIXIA XU[1] and Robert Buchanan[2]
[1]University of Maryland, Department of Nutrition and Food Science, College park, MD, [2]University of Maryland, Department of Nutrition and Food Science and Center for Food Safety and Security Systems, College Park, MD

❖ ***Developing Scientist Competitor***

Introduction: Preharvest testing for pathogens and indicator microorganisms is increasingly used to enhance the microbial safety of fresh produce. Traditional sampling plans assume sample collectors have no information of potential contamination sources. Knowledge of factors that could lead to nonrandom contamination could potentially increase the effectiveness of preharvest sampling programs.

Purpose: The goal of this study was to use mathematical modeling to determine the impact of including a portion of the samples based on the sampler's knowledge of risk factors. The performance characteristics of sampling plans that include such "samples of opportunity" (SOO) were compared to that of traditional preharvest sampling plans.

Methods: Computer simulations were performed to compare the relative effectiveness of random, stratified-random, and z-pattern vs. SOO sampling. The SOO sampling reserved two thirds of samples to be taken from identified high-risk areas within a field. These evaluations assumed the contamination in the field was nonrandom, with three contamination scenarios being evaluated: animal house nearby, power line above the field, and field partially exposed to floodwaters. The simulation modeling tool allowed a large number of field contamination scenarios to be generated and evaluated systemically.

Results: The detection probability for a nonrandomly contaminated preharvest field (five by six plots with nine subplots per plot (total of 270 subplots)) using random, stratified-random, and z-pattern sampling plans was 0.30±0.11, 0.32±0.11, 0.32±0.17, respectively. The SOO sampling plan had a detection probability of 0.61±0.25. The detection probability of SOO was 96% higher than other sampling plans (P<0.001). However, if the assumption of contamination source is incorrect, detection probability of SOO drops to 0.33±0.23, which is not significantly different than the other sampling plans.

Significance: This study provided a mathematical approach for evaluating the effectiveness four preharvest sampling plans, and suggested that having the knowledge of the contamination source in the field would improve effectiveness of sampling.

T8-01 Comparison of Alternative Sanitizers to Chlorine Disinfection for Reducing Foodborne Pathogens in Avocados, Melon, Citrus, and Cucumbers

ADRIAN SBODIO, David Hill, Jeremy Roland, John Alaniz and Trevor Suslow

University of California-Davis, Davis, CA

Introduction: Chlorine has been the primary food safety disinfectant used in water treatment; however, limited efficacy and byproduct formation has led to a search for more effective, safer alternatives.

Purpose: The efficacy of chlorine (NaClO), peroxyacetic acid (PAA), and Safe Zone as antimicrobial water treatments were compared.

Methods: Hass avocados, cantaloupe, melons, and navel oranges were spot inoculated with attenuated and pathogenic *Salmonella* spp., stored at 15 or 10°C (citrus) for four days postinoculation (DPI), and treated under simulated recirculating wash water conditions. Simultaneously, uninoculated and inoculated fruits (2:1 ratio) were treated for 0.5, 1, 5, and 10 min with either chlorine (NaClO), peroxyacetic acid (PAA), or SafeZone. Quantitative and qualitative assessments for *Salmonella* were performed before and after disinfection. Lemons, mandarins, and cucumbers were inoculated and treated with SafeZone, only. The target dose before disinfection was five log CFU/avocado, melon and citrus, and six log CFU/cucumber.

Results: *Salmonella* counts in avocados and oranges treated with SafeZone, PAA, and chlorine declined to 2 to 1.4 log CFU/fruit after one minute, with 60 to 100% positive in chlorine and PAA treated avocados, and 0 to 50% in SafeZone treated avocados and oranges. SafeZone, PAA, and chlorine treated melons resulted in *Salmonella* reductions of three, four, and five log CFU/melon circle, respectively. In SafeZone treated lemons and mandarins, *Salmonella* counts declined to the limit of detection after 10 min, with 10% and 73.3% positives, respectively. In SafeZone treated cucumbers, *Salmonella* reduction of two to four log was observed with intact samples during inoculation; however, almost no decline was detected when damaged or bruised. Cross-contamination was observed in uninoculated avocados treated with chlorine and PAA (5% and 13%), while prevented in SafeZone treated avocados and citrus. Chlorine and PAA treated melons had the highest rates of cross-contamination (77 to 100%); SafeZone decreased these (<16.7%).

Significance: Comparative evaluation demonstrated SafeZone as a practical antimicrobial water treatment, achieving better results than chlorine and PAA.

T8-02 Enteroaggregative *Escherichia coli*: Predominant Diarrheagenic *Escherichia coli* Pathotype among Irrigation Water and Food Sources

Matthew Aijuka[1], Araceli Santiago[2], Jorge Girón[3], James Nataro[4] and **ELNA BUYS**[1]

[1]University of Pretoria, Pretoria, South Africa, [2]University of Virginia, Charlottesville, VA, [3]University of Virginia, Charlottsville, VA, [4]University of Virginia School of Medicine, Charlottesville, VA

Introduction: Diarrheagenic *Escherichia coli* (DEC) has been implicated in foodborne outbreaks worldwide and have been associated with childhood stunting in the absence of diarrhea. Infection is common, but the routes of transmission have not been determined.

Purpose: This study was undertaken to determine the most prevalent pathotypes, as well as associated foods and environmental sources. The information gathered will help to provide better guidance to various stakeholders in ensuring food safety and public health.

Methods: PCR, partial gene sequencing, and cell adherence were used to characterize 205 *E. coli* strains, previously isolated from milk (118), irrigation water (48), irrigated lettuce (29), and coleslaw (10) in South Africa. The results of the characterization were used to group the strains, based on four diarrheagenic *E. coli* pathotypes; enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC).

Results: Using a combination of molecular and phenotypic assays, only EAEC (37%, 17 of 46) and EIEC (4.3%, 2 of 46) were identified in the samples. PCR and partial gene sequencing initially confirmed EAEC (2.4%, 5 of 205) virulence gene determinants (*aatA* and *aaiC*) as the only pathotype. Phylogenetic analysis based on identity of *aatA* and *aaiC* genes, in strains from this study to those in Genbank, showed close relatedness to foodborne and uropathogenic strains. Human strains clustered differently from environmental strains, suggesting a potential role for use of virulence gene determinants in source tracking.

Significance: EAEC may be the leading cause of food- and waterborne enteric infection in South Africa. Additionally, sole use of molecular based methods targeting virulence gene determinants may underestimate numbers, especially among heterogeneous pathogens such as EAEC.

T8-03 Evaluating the United States Food Safety Modernization Act Standard for Microbial Quality of Agricultural Water for Produce Growing

KATHLEEN VAZQUEZ[1], Arie Havelaar[1], Zeynal Topalcengiz[2], Rafael Muñoz-Carpena[1] and Michelle D. Danyluk[3]

[1]University of Florida, Gainesville, FL, [2]Muş Alparslan University, Muş, Turkey, [3]University of Florida, Lake Alfred, FL

Introduction: The Food Safety Modernization Act (FSMA) brought new regulations concerning the bacteriological quality of irrigation water used for fresh produce. Microbial criteria in the form of geometric mean (GM, 50th percentile of a lognormal distribution) and statistical threshold value (STV, 90th percentile of a lognormal distribution) calculated from 20 samples of *Escherichia coli* concentrations over a two to four year period are used to evaluate water quality.

Purpose: The purpose of this study was to investigate (i) how well lognormal distributions fit empirical data; (ii) how estimates of microbial criteria are affected by detection limits; (iii) if 20 samples are sufficient to characterize the water quality; (iv) how sensitive the microbial criteria are to shifts in water quality; and (v) the predictive ability of *Escherichia coli* for the presence of *Salmonella* spp.

Methods: This study used 540 samples from six irrigation ponds measuring *E. coli* concentrations, *Salmonella* spp. presence, turbidity, and other physicochemical parameters. Objectives were analyzed by (i-ii) fitting distributions to data using maximum likelihood estimation, while considering censoring; (iii) analyzing data subsets to simulate limited sampling; (iv) simulating shifts in water quality using generated data and measuring the time until microbial criteria reflect the shift; (v) using logistic regression.

Results: Lognormal distributions provided an adequate fit and accounting for censoring due to detection limits increased the spread of fitted distributions. Due to high variability in *E. coli* counts, 20 samples were not sufficient to characterize the water quality and sudden shifts in water quality were

not detected using the prescribed sampling scheme for as many as six years. *Escherichia coli* was found to be an adequate predictor of *Salmonella* spp. presence, with turbidity as an additional significant variable.

Significance: When bacteriological quality of irrigation ponds has high variability, as in this study, alternative approaches to ensuring water quality should be considered.

T8-04 Methods for Identifying and Mitigating Vulnerable Nodes in a Food Process

Clint Fairow[1], **CAROL BREVETT**[2], Jessica Cox[3], Luke Bicknese[4], Penny Norquist[5], Ted Steinmann[4], Lehman Waiswisz[1] and Joseph Zarzycki[6]
[1]ADM, Decatur, IL, [2]Leidos, Gunpowder, MD, [3]DHS, Washington, DC, [4]University of Minnesota, Minneapolis, MN, [5]FPDI, Saint Paul, MN, [6]CSAC, APG, MD

Introduction: The Department of Homeland Security (DHS) quantifies the consequences and risks of chemical and biological terrorism attacks on the national food supply chain via the biennial Chemical Terrorism Risk Assessment (CTRA), and Biological Terrorism Risk Assessment (BTRA). The Food Safety Modernization Act (FSMA), which became law in 2011, addresses the use of mitigation strategies to protect food from intentional adulteration by microbes or chemicals. Hence, calculating the health effects of chemical and biological adulterants in foods, is an area of common interest between CTRA, BTRA, FSMA, and industry.

Purpose: The CTRA and BTRA consider national and local impacts; their Desktop Tool software contains useful agent data, but the embedded food processes are too generic for one company to use for mitigation of risk. A collaboration between CSAC, ADM, and FPDI to address industry needs on a per-process basis resulted in the stand-alone, downloadable Intentional Adulteration Assessment Tool (IAAT) software package.

Methods: The health effects of adulterant in a batch of food were compared using CTRA and BTRA Desktop Tools and IAAT. Food processing details were entered into IAAT and the effect of various mitigation strategies on the health effects were calculated.

Results: CTRA and BTRA report the health effects on a national or city level, and the recall of a product is a major variable in the total number of illnesses. The IAAT gives the potential health effects for the batch size, based upon the locus of adulterant addition within the process. The IAAT gives the producer the ability to restrict physical access to vulnerable locations, and hence the opportunity to analyze and remove risk from the manufacturing process.

Significance: Food producers can use the IAAT to analyze the risk within their own unique processes and focus mitigation strategies where needed.

T8-05 Toward an Extended Food Safety Culture Model: Studying the Moderating Role of Burnout and Job Stress, the Mediating Role of Food Safety Knowledge, and Motivation in the Relation between Food Safety Climate and Food Safety Behavior

ELIEN DE BOECK, Anneleen V. Mortier, Liesbeth Jacxsens, Lisa Dequidt and Peter Vlerick
Ghent University, Ghent, Belgium

Introduction: Previous research demonstrated the influence of food safety climate on the food safety output of food companies on an organizational (company) level. At the individual level, the relation between food safety climate and employees' food safety behavior still has to be unraveled. Therefore, the conceptual food safety culture model of DeBoek, Jacxsens, Bollaerts, and Vlerick (2015) was expanded by introducing food safety behavior, knowledge, motivation, burnout, and job stress of the individual employees in the organization.

Purpose: In the present study the relationship between food safety climate and food safety behavior was investigated. Food safety knowledge and motivation were proposed as mediators, explaining the relationship between climate and behavior. Additionally, job stress and burnout were proposed as moderators, influencing the strength of this relationship.

Methods: This conceptual model was tested through statistical analysis with data (n=85) collected from two Belgian vegetable processing companies through self-assessment surveys.

Results: A positive relationship between food safety climate and employees' behavior was found. Mediation analysis showed that knowledge is a partial mediator between food safety climate and compliance, participation, and behavior, which means that knowledge cannot fully explain this relationship. Motivation is a partial mediator between food safety climate and compliance and behavior, only. The moderation effect between job stress and burnout was not confirmed. These results demonstrate the direct (without mediation) and indirect effect (through motivation and knowledge) of food safety climate on employees' behavior and illustrated the key role of employees' behavior and well-being for governing food safety in a company.

Significance: This study suggested that human factors might impact the implementation and follow-up of a food safety management system and recommended a more human behavioral approach for the food safety management in food companies.

T8-06 Assessment of Nigerian Food Inspection Capabalities, Practices, and Procedure

CHRISTINE IKPEME-EMMANUEL
University of Calabar, Calabar, Nigeria

Introduction: The new global environment for food trade places considerable obligations on both importing and exporting countries to strengthen their food control systems. A robust food safety control system requires clear inspection policy and procedure that are applied by food inspectors, who are well trained not only to apply these procedures, but also to act as quality assurance advisors and extensions officers to the food industry. The global trend toward food inspection is the application of risk-based inspection, which is based on a preventative approach. High rates of foodborne diseases and food rejection at point of export have been reported for Nigeria. Consequently, there is a need to assess the food inspection practices and procedures of Nigeria with the aim of strengthening any identified weakness.

Purpose: This study was undertaken to assess Nigeria's food inspection practices and procedures that would support its review for alignment with global best practices.

Methods: Methodology involved consultation with the seven national agencies with food jurisdictions, using the face–to–face interview technique. Data was collected on a structured closed-ended questionnaire instruments adapted from the FAO quick guide to assess capacity building needs for food inspection.

Results: The results indicated lack of the following: dedicated food inspectors, clearly defined criteria for employment as food inspectors, quality and quantity of food inspectors at relevant sectors from farm to table, unified inspection manual or guidelines and use of a risk-based inspection system, and high rates of noncompliance to regulations by operators. Findings indicated the need to develop an appropriate food safety inspection program, procedures, and operations, based on risk, that will cater adequately to all food sectors and align with best practices.

Significance: A strengthened food inspection system would reduce the risk of foodborne diseases and also increase Nigerian's access into the international market.

T8-07 USDA-FSIS Food Defense and Recall Preparedness Scenario-based Table Top Exercise Tool

BRYAN NORRINGTON
U.S. Department of Agriculture - FSIS, Washington, DC

Introduction: The Food Safety and Inspection Service (FSIS) expects to release a modernized version of its Food Defense and Recall Preparedness Scenario-Based Table Top Exercise *Tool*, now called the *Food Defense Preparedness and Recall Exercise Package* (FD-PREP), in mid-to-late 2017.

Purpose: Originally released in 2013, the Exercise Tool was developed to enhance awareness of the importance of protecting the food supply from intentional adulteration, ensure food defense practices and mitigations were effective, documented and understood, and to support industry efforts to test and maintain effective written recall procedures.

Methods: Designed primarily for small and very small establishments, this discussion-based Exercise Tool has been updated to improve usability and provide three new scenarios that reflect current issues.

Results: Each scenario now addresses a unique incident type and are applicable to all establishments, regardless of the commodity they produce. The Tool will help establishments test and validate their food defense and recall plans.

Significance: The modernized Exercise Toolkit will include Homeland Security Exercise and Evaluation Program (HSEEP) compatible exercise documents for use by local governments or industry associations that might conduct an exercise for establishments in their functional area. These documents will ensure conformance with established guidance in exercise program management, design, evaluation, and improved planning. HSEEP guidance and exercises may qualify for Federal or state grant funding.

T8-08 Challenging the Food Emergency Response Network with the Detection of a Select Agent in Foods

AMIE MINOR[1], Christian Robinson[2], Zachary Kuhl[2], Justin Ferrell[2] and Brenda Keavey[1]
[1]West Virginia Department of Agriculture, Charleston, WV, [2]West Virginia Department of Agriculture, Charleston, WV

Introduction: Abrin is an extremely toxic ribosomal inactivating protein commonly found in the tropical plant, Abrus precatorius. Abrin toxicity inhibits the synthesis of cell proteins, causing severe illness and cell death. Although the use of abrin has not been documented in a bioterrorism event, its lethality, availability, ease of dissemination, and lack of antidote make it a dangerous foodborne contaminate with the potential for bioterrorism activity. Food proficiency testing programs for many select agents, including abrin, are not readily available. Food defense laboratories within the network are required to prove competency prior to providing testing capabilities for surveillance and surge testing of abrin toxin in foods.

Purpose: The objective of this activity was to provide a challenging proficiency test to the Food Emergency Response Network for the detection of abrin toxin in foods.

Methods: Temperature and stability studies were conducted with abrin toxin in hot dogs for three weeks, prior to shipping of samples. Twelve participating laboratories throughout the country received eight unknown samples, fortified with abrin toxin (varying from 500 to 1,000 ng/g), as well as blanks. Laboratories analyzed samples following the FERN.MIC.0021 method, incorporating a toxin extraction via centrifugation in conjunction with the Tetracore Abrin ELISA detection kits.

Results: All laboratories (12 of 12) reported the qualitative sample results with 100% proficiency (96 of 96). Eleven of twelve (11 of 12) participating laboratories, additionally, reported quantitative absorbance values for each sample analyzed. All z score calculations for individual laboratory results were acceptable $|z|<3$, based on the total comparison group's mean consensus calculated absorbance and standard estimation of variation.

Significance: The data generated from this activity was utilized by state laboratories across the country to provide evidence of individual competency for the detection of abrin in foods. This method was used in 2016 for a national biodefense surveillance activity for abrin detection.

T8-09 The Beneficial Impact of Restaurant Letter Grade Posting on the Occurrence of *Salmonella*

Ana Ebbert and **CRAIG HEDBERG**
University of Minnesota, School of Public Health, Minneapolis, MN

Introduction: Rates of *Salmonella* infection in the United States have not changed over the past 20 years. Restaurants are frequent settings for *Salmonella* outbreaks and are risk factors for sporadic *Salmonella* infections. A marked decline in foodborne illness hospitalizations was reported in Los Angeles County following the introduction of restaurant grade postings at the point of service.

Purpose: We sought to evaluate the potential for posting restaurant inspection letter grades at the point of service to reduce the occurrence of *Salmonella*.

Methods: We identified state and local jurisdictions that implemented grading of restaurant inspections wih posting of letter grades at the point of service. We, also, identified jurisdictions that posted inspection results other than letter grades at the point of service. For each jurisdiction, we obtained counts of *Salmonella* infections reported in the jurisdiction for the two years before the posting practice was implemented, for the year of the change, and for the two years after the change. Observed rates were compared to aggregate FoodNET *Salmonella* data.

Results: Six jurisdictions were identified that implemented letter grade posting of restaurant inspection results and had available *Salmonella* data. Six jurisdictions were identified that posted other inspection results. The mean rate of *Salmonella* in the letter grade jurisdictions declined from 15.0 cases per 100,000 population (two years before the change) to 11.2 cases per 100,000 population (two years after the change). These rates were significantly lower than comparable FoodNET rates for the year of change and the two following years ($P<0.05$). In jurisdictions that posted other forms of results, the rates did not change.

Significance: Posting restaurant inspection results at the point of service in the form of a letter grade reduced reported *Salmonella* cases in the jursdiction and, therefore, warrants broader use.

T8-10 Safe Food for Canadians Regulations

RICHARD ARSENAULT and Kevin McBain
Canadian Food Inspection Agency, Ottawa, ON, Canada

Introduction: Canada has one of the best food safety systems in the world. However, continuous improvement is needed to maintain Canadians' access to safe food.

Purpose: The Safe Food for Canadians Act, which received Royal Assent in November, 2012, establishes a modern and robust legislative framework for the safety of food commodities sold to Canadians. It also provides ongoing reviews of the federal food safety system in Canada.

Methods: The Act focuses on improving food safety oversight, streamlining and strengthening legislative authorities, and enhancing international market opportunities for Canadian industry. With an anticipated coming-into-force date of 2018, the Canadian Food Inspection Agency will publish draft regulations in early 2017 to support the Act.

Results: Consistency of approaches with international jurisdictions has been a priority in developing the regulations.

Significance: This presentation will be of interest to United States. and international food entities with business relationships in Canada. It will also serve to inform them of what the Safe Food for Canadians Regulations could mean for their businesses.

T8-11 Valuing the Burden of Foodborne Illness in Regulatory Analysis

ANGELA LASHER

U.S. Food and Drug Administration, College Park, MD

Introduction: As required by Executive Order 12866 and strengthened by Executive Order 13563, regulatory agencies are required to perform impact analyses to determine the societal costs and benefits of any proposed regulatory action. Since the early 1990s, FDA has made use of the Quality of Well-Being scale and then the EuroQol- 5 Dimensions scale to value the full cost to victims of foodborne illness. Over this time period, the value applied to this cost, in the form of a value of a statistical life (VSL), has varied.

Purpose: The purpose of this study was to demonstrate how evolving methods and changes to the VSL used can affect the net benefits of proposed regulatory actions in the realm of food safety.

Methods: Acceptable values for a statistical life can range from $1 million to more than $20 million. New Department of Health and Human Services Guidelines suggest low, central, and high VSL estimates of $4.5 million, $9.6 million, and $14.6 million in 2016 dollars, respectively. Results suggest that Executive Branch authoritative bodies such as the Office of Management and Budget (OMB) would be well-served to update its guidance and best practices, using current or new methods, for choosing the appropriate value of a statistical life for agency regulatory purposes.

Results: Results of previous regulatory actions were examined with changing values of statistical life. These hypothesized changes in VSL allowed for a reexamination of the net benefits of previous food safety regulations.

Significance: The range and mean value of statistical life presented in regulatory impact analyses can play a significant role in the expected societal benefit, or burden, of a regulation.

T8-12 Assessment of the Presence of Foodborne Physyical Hazards in South East Europe Using Data from EU Rapid Alert System for Food and Feed

ANDREJA RAJKOVIC[1], Danijela Jankovic[2] and Ilija Djekic[3]

[1]Ghent University, Ghent, Belgium, [2]University of Belgrade-Faculty of Agriculture, Department of Food Safety and Food Quality Management, Belgrade, Serbia, [3]University of Belgrade-Faculty of Agriculture, Belgrade, Serbia

Introduction: The European Union (EU) established the Rapid Alert System for Food and Feed (RASFF) database to capture information in respect to various food safety and food fraud aspects. Foreign bodies are considered to be objects, not typically present in food, that result in injuries, diseases, or psychological trauma. Presence of this hazard has not, in general, been explored and is often neglected in research and food safety trainings.

Purpose: The purpose of this paper was to analyze the presence of foreign bodies, during the extended period of 12 years. It provides information on 731 incidents of foreign matter contamination deployed in terms of types of foreign bodies and food products engaged, from 13 countries of South and East Europe.

Methods: All notifications recorded in the RASFF database from countries of SE Europe were extracted for January 01, 2004 to December 31, 2015. Data regarding year of notification, product category, country notifying, and foreign body were further processed. The chi-square test for association elucidated relationships between regions and types of foreign body or food industries.

Results: Analysis of foreign bodies noted that the top three materials were (i) pests (82.1%), (ii) metal (4.6%), and (iii) glass (3.3%). Chi-square confirmed a statistically significant association between the types of foreign bodies and regions in which they occur (X^2 = 79,870, P<0.05). Analysis of food categories involved in these notifications revealed that the top three product categories were (i) nuts, nut products, and seeds (35.4%), (ii) fruit and vegetables (27.2%) and (iii) bakery and confectionery products (12.0%). Chi-square confirmed a statistically significant association between the types of food industries and regions in which they occur (X^2=130,218; P<0.05).

Significance: Results from a wide range of foreign bodies demonstrates the most dominant types of foreign bodies present in food products, from the two European regions, and provide clear realtionships between types of foreign bodies, types of food industries, and geographic regions.

T9-01 Spores under High-pressure High-temperature Processing Conditions

Brigitte Cadieux[1], Hamed Vatankhah[1], **LAWRENCE GOODRIDGE**[1], John W. Austin[2] and Hosahalli S. Ramaswamy[1]

[1]McGill University, Ste-Anne-de-Bellevue, QC, Canada, [2]Health Canada, Ottawa, ON, Canada

Introduction: Spores produced by *Clostridium botulinum* are highly resistant to heat and require long processing times to become inactivated. This can lead to the deterioration of food quality. High Pressure High Temperature (HPHT) processing is an alternative technology that can produce safe foods while maintaining the highest quality of the product.

Purpose: The goal of this study was to evaluate the destruction kinetics of group II (nonproteolytic) *C. botulinum* spores under various HPHT processing conditions.

Methods: Group II *C. botulinum* spores produced by eight strains from different serotypes (B, E, F) were suspended in ACES buffer (10^6 spores/ml) and individually processed at 600 MPa, 80°C. To determine the *D*-value, spore survival was enumerated by plate count on McClung Toabe agar with egg yolk and the plates were incubated anaerobically for seven days at 25°C. Spores exhibiting the highest resistance were further processed at 550 and 650 MPa at 80°C. The *z*-value as a function of pressure was, later, calculated. Each treatment was repeated in triplicates.

Results: Spores from *C. botulinum* type F strains exhibited the highest *D*-values (3.2 to 7.3 minutes) upon processing at 600 MPa at 80°C, followed by type B strains (2.4 to 5.8 minutes) and type E strains (2.6 to 2.7 minutes). Strains 610F, KAP-B-8 and E Russ showed the highest resistance within their respective type and were selected for subsequent experiments. The *D*-values of these three strains increased at 550 MPa (7.4 to 36.2 minutes) and decreased at 650 MPa (2.0 to 6.0 minutes). Based on the *z*-value, strains KAP-B-8 and 610F produced spores which were the most resistant to HPHT (128.2°C and 153.8°C, respectively), while spores from E Russ were the least resistant (200.0°C).

Significance: HPHT is an effective method for destroying *C. botulinum* spores and could potentially be used as a processing method to achieve commercial sterility while maintaining the quality of food products.

T9-02 High-pressure Processing and Cultures: The Right Combination to Produce Safe Semi-dried Italian Fermented Sausages without Taste Compromise

Elena Brugnoli[1], Jenny Triplett[2] and **VERONIQUE ZULIANI**[3]
[1]CHR HANSEN, Parma, Italy, [2]CHR HANSEN, Milwaukee, WI, [3]CHR HANSEN, Arpajon, France

Introduction: *Listeria monocytogenes* and *Salmonella* spp. are the two main pathogenic bacteria that pure pork sausages manufacturers must address. Here, the combination of a starter culture including a bacteriocin producing strain and high-pressure processing (HPP) were tested to obtain a sufficient log reduction during a typical Milano process.

Purpose: Challenge tests were performed to evaluate the *Salmonella* and *Listeria* log reduction during the Milano process and after the HPP treatment.

Methods: A standard 100% pork batter was obtained from an Italian producer. The meat batter was inoculated with a cocktail of *Listeria innocua* and *Salmonella* spp. and properly dosed with the starter culture before being stuffed into a 90 mm casing. Sausages were then ripened at 72/75°F (minimal pH 5.35 in 72 h/ final pH 6.0) and dried until reaching a 30% weight loss (water activity ~0.91). HPP (6,000 Bar/5 minutes/15°C) was applied after drying. *Listeria*, *Salmonella*, Lactic Acid Bacteria, coagulase-negative *Staphylococcus*, and *Pediococcus* concentrations were measured just after stuffing, at the end of the drying step, and after HPP treatment.

Results: The starter culture maintained high concentration during the salami process, as well as after HPP Treatment. The milano process, even with a gradual pH drop and slow drying process, which typically supports higher pathogenic risk, still allowed a 3.2- and 2.3-log reduction, respectively, for *Salmonella* spp. and *L. innocua*. After HPP, a total of six- and five-log reduction, respectively, for *Salmonella* spp. and *L. innocua* were achieved.

Significance: These results suggested that the combination of starter culture, including a bioprotective strain, and HPP significantly reduce pathogenic bacteria contamination. This allowed maintenance of the traditional Milano process to obtain typical flavor and achieve more food safety.

T9-03 Effect of Pressure, Spoilage Microbiota, and Antimicrobials on Survival and Post-pressure Growth of *Listeria monocytogenes* on Ham

JANUANA TEIXEIRA, Lynn McMullen and Michael Gänzle
University of Alberta, Edmonton, AB, Canada

Introduction: Pressure treatment of ready-to-eat (RTE) meats extends shelf life and reduces risks associated with *Listeria monocytogenes*. However, pressure reduces numbers of *Listeria* on ham by less than five log CFU/g and pressure effects on spoilage organisms are poorly documented.

Purpose: This study aimed to investigate the impact of pressure and meat spoilage microbiota, with or without antimicrobials, on survival of *Listeria* after refrigerated storage.

Methods: Ham was inoculated with a five-strain cocktail of *L. monocytogenes*, alone or together with a cocktail of spoilage organisms (*Leuconostoc* spp., *Lactobacillus sakei*, *Carnobacterium maltaromaticum*, and *Brochothrix thermosphacta*). Products were treated at 500 MPa at 5°C for one or three minutes, with or without nisin or rosemary extract. Differential enumeration of cells was done after pressure treatment and after four weeks of refrigerated storage. Experiments were performed in triplicate.

Results: Treatment of *Listeria* for one or three minutes reduced counts by 1.05±0.39 and 2.08±0.33 log (CFU/g), respectively; inactivation of spoilage microbiota was comparable. Counts of *Listeria* increased by three and one log CFU/g during refrigerated storage after one or three minutes of treatment, respectively. The presence of spoilage microbiota did not influence inactivation of *Listeria* but prevented growth of *Listeria* after refrigerated storage. The addition of rosemary extract did not influence inactivation of *Listeria* or the spoilage microbiota, or growth of microorganisms during storage. The combination of nisin with pressure treatment for three minutes reduced counts of *Listeria* and spoilage microbiota by greater than five log CFU/g; after four weeks of storage counts were below the detection limit.

Significance: In conclusion, pressure application alone did not eliminate *Listeria* or spoilage microbiota on RTE ham; however, survival of spoilage organisms prevented growth of *Listeria* on pressure treated ham. Overall, this research furthers our understanding of the impact of pressure on lethality of spoilage organisms and *Listeria*.

T9-04 Quality and Safety Evaluation of Striped Catfish Processing Byproducts in Vietnam

NGUYEN. V. NGUYEN[1], Thao. T.H. Nguyen[2], Trung. D. Le[2] and Men. T. Dinh[2]
[1]Research Center for Aqua-Feed Nutrition and Fishery Post-Harvest Technology, Ho Chi Minh, Viet Nam, [2]Research Center for Aqua-Feed Nutrition and Fishery Post-Harvest Technology, Ho Chi Minh, Viet Nam

Introduction: The Vietnam striped catfish industry has rapidly developed and, in recent years, has processed over one million tonnes per year in recent years. Approximately 30% of the total production is in fillet form and the remainder (70%) is byproducts, including head, bones, fin, skin, fat, and organs. Byproducts are normally processed within 24 hours so that the evaluation of lipid oxidation and bacterial contamination during storage is necessary to control the deterioration and environmental impacts.

Purpose: This study evaluated the quality of byproducts during preservation.

Methods: Byproducts from five different catfish processing factories in Mekong Delta were randomly sampled to investigate the quality evolution through lipid oxidation and microbiological count (n=20). Belly fat and byproducts analyzed to determine the fluctuation of fat and microbiological count after the fillet process and after 24 hours of preservation. Crude fat, free fatty acid and acid value of belly fat were determined and bacteria consisted of coliforms, *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus, Clostridia* spp. Total yeasts and molds were counted. Data were statically analyzed by using SPSS version 18 with statistical significance (P<0.05)

Results: The results indicated that after 24 hours of preservation, the fat quality tended to reduce. This, in turn, resulted in increases in the free fatty acids from 1.42±0.10 to 1.79±0.03% and acid value from 2.83±0.20 to 3.56±0.07 mg KOH/100 g. Coliform bacteria counted from 1,100 to 290,000 (MPN/g), *Clostridia* spp. from 0 to 1,100 CFU/g, total counts of yeasts and molds presented from 0 to 500,000 CFU/g, while *S. aureus* numbered from 0 to 10 CFU/g and *E.coli* from 0 to 7.4 MPN/g. *Salmonella* bacteria were not found.

Significance: The storage time and conditions of byproducts should be carefully controlled to avoid reducing lipid quality and microbiological contamination.

T9-05 Efficacy of a High-intensity Preconditioner for Reducing *Enterococcus faecium* Populations as a Non-pathogenic *Salmonella* Surrogate in Kibble-style Pet Food

NICHOLAS SEVART, Tiya Zhou, Sajid Alavi, Charles Stark and Randall Phebus
Kansas State University, Manhattan, KS

Introduction: Illnesses and product recalls due to *Salmonella* contamination have raised concerns about pet food safety. Published research findings are lacking regarding validation of dry kibble-style pet food processes.

Purpose: This study validates the efficacy of a high-intensity-preconditioner (HIP) for controlling *Enterococcus faecium*, a nonpathogenic surrogate for *Salmonella* spp. in heat treatment validations, in a generic dog food formulation.

Methods: Dry pet food ingredients (~227 kg) were spray inoculated with *E. faecium* (~5.8 log CFU/g; 30 min attachment period). Batches of pet food were treated through a HIP system using three different treatment parameters: A) 68°C for 154 s, B) 68°C for 65 s, or C) 90°C for 65 s. *Enterococcus faecium* populations were enumerated by direct plating onto selective and injury-recovery agars, before and after HIP treatments, to quantify process lethality.

Results: Treatment B resulted in the least microbial lethality ($P \leq 0.05$), achieving approximately a 1.5 log CFU *E. faecium*/g reduction. Treatments A and C were statistically different when comparing selective media counts, achieving 3.9 and 5.1 log CFU/g reductions, respectively. However, these two treatments were similar ($P > 0.05$) when comparing injury recovery counts (3.4 and 3.5 log CFU/g reductions, respectively), indicating the presence of a higher population level of sub-lethally injured cells resulting from the higher temperature, shorter exposure time provided by treatment C.

Significance: Preconditioning of raw ingredient formulations is a common thermal treatment prior to extrusion processes in the generation of kibble-style pet foods. The present study validated the efficacy of three HIP processes for reducing *E. faecium*. This study was indicative of the lethality effect expected for *Salmonella* spp. since *E. faecium* was used as a surrogate organism in similar heat treatment validations of foods, and generally is slightly higher in its thermal tolerance. Pet food processors can use this information to help define critical control points when using an HIP system.

T9-06 Determination of Acrylamide in Fried Potato Chips and the Impact of Various Treatments on Acrylamide Formation during Frying

ASAD AMJAD[1], Muhammad Nasir[1], Frasat Rizwan[1], Mateen Abbas[2], Muhammad Shahbaz[3], Abdul Muqeet Khan[2] and Umar Bacha[4]
[1]University of Veterinary & Animal Sciences, Lahore, Pakistan, [2]Quality Operations Laboratory (QOL), University of Veterinary & Animal Sciences, Lahore, Pakistan, [3]Mawarid Food Company - KSA (Pizzahut, Taco Bell), Riyadh, Saudi Arabia, [4]University of Management & technology, Lahore, Pakistan

Introduction: Acrylamide is well known industrial chemical primarily used in the gel electrophoresis technique and waste water treatment plant in polymer form. Acrylamide generates in heat treated food products, mainly in carbohydrate rich foods such as potato and cereal products via Maillard reaction.

Purpose: The current study was conducted to determine the acrylamide concentration in various potato chips samples from fast food chains and some commercially available brands. The objectives of this study were to determine the acrylamide content in fried potato chips available in the local market of Lahore and to minimize the concentration of acrylamide formation in fried potato chips by applying various treatments.

Methods: Twenty samples were analyzed from fast food chains, commercially available brands, local vendors, and homemade potato chips. Three treatments were applied to minimize the acrylamide concentration in potato chips. Twelve samples were treated, in the laboratory, in oil (170°) for two minutes. Extraction and HPLC analysis were performed to determine acrylamide concentrations.

Results: Samples collected from local vendor contained a high level of acrylamide (2,429 ppb), followed by homemade potato chips (1,460 ppb). Fast food chain B (FFC B) contains high level of acrylamide (559 ppb) as compared to fast food chain A (FFC A; 255 ppb). Commercially available brand contain the lowest concentration of acrylamide (60 ppb). Three treatments were applied to reduce or minimize acrylamide in fried potato chips. Par fried treated potato chips contained 73 ppb; prepared frozen potato chips, 144 ppb; while par frying prepared frozen potato chips contains lowest acrylamide level, 44 ppb.

Significance: The present study illustrated that carbohydrate and amino acids are the main source of producing acrylamide in fried, heated, or processed food stuffs (starchy foods). Acrylamide contents can be reduce by decreasing precursor quantities (reducing sugar and amino acids) that are thought to be responsible for the Maillard reaction.

T9-07 Design of a Low Concentration Sodium Nitrite Meat Product by Including *Ullucus tuberosum* from Ventaquemada, Boyacá, Colombia

LAURA CHAVES
Pontificia Universidad Javeriana, Bogotá, Colombia

❖ ***Developing Scientist Competitor***

Introduction: Sodium nitrite (SN) is an antimicrobial food additive widely used by the meat industry for the manufacture of sausages. SN is transformed into N-nitrosamines, which are known to be carcinogenic and mutagenic. Therefore, several alternatives have been explored to find compounds capable of replacing SN and ensuring the microbiological stability of the product. It has been demonstrated that the incorporation of plant extracts allows a reduction in the amount of SN added. Ullucus tuberosum (UT) is a Colombian tuber that has been suggested to have antimicrobial properties. In addition, it has been established that the inclusion of UT in food products does not cause a significant modification of the sensory profile of the product.

Purpose: The objective of this study was, therefore, to design a meat product (sausage), including UT to obtain a low concentration SN sausage, with a negligible modification of the sensory profile, and a improved microbiological stability.

Methods: Sausage formulations, with the addition of 4.5% (w/w) UT and SN concentrations ranging from 50 to 200 mg/kg of meat emulsion, were produced. Then, the microbiological stability (shelf life measured by the aerobic mesophilic count) and the sensory profile [affective hedonic test (AHT) with 73 untrained panelists] of all products were evaluated. The control corresponded to the sausage formulation with no addition of UT and 200 mg/kg SN.

Results: On one hand, the sausage formulation containing UT and 100 mg/kg SN exhibited the same shelf life as compared to the control (20 days). On the other hand, AHT revealed that the sausage containing UT and 100 mg/kg SN exhibited the best sensory profile of all of the tested sausages.

Significance: The importance of this study lies is the obtention of processed foods with improved microbiological, sensory, and nutritional quality, which encourage the use of ancestral plant species that are marginalized.

T9-08 Inactivation of Pathogenic Bacteria in Ice Using an Ultraviolet C Light-emitting Diode

SUGURU MURASHITA, Shuso Kawamura and Shigenobu Koseki
Hokkaido University, Sapporo, Japan

❖ ***Developing Scientist Competitor***

Introduction: Ice is widely used in the food industry; however, it can be contaminated with microbes, and thus can cause food poisoning. Ice-contaminating microorganisms originate from poor quality source water and/or a lack of hygiene during production or handling. Direct microbial inactivation of ice could ensure its microbiological safety. Thus, we studied direct inactivation of microorganisms in ice using an ultraviolet C light-emitting-diode (UVC-LED).

Purpose: The purposes of this study were to compare the performance of UVC-LEDs with conventional UV lamps and to investigate the inactivation effect of UVC-LED on pathogenic bacteria in ice.

Methods: Four strains of *Escherichia coli* O157:H7, five strains of *Salmonella* Typhimurium, and six strains of *Listeria monocytogenes* were used. One milliliter of each bacterial suspension was added to 24 ml of distilled water in ice cube tray (length: 30 mm, width: 30 mm, thickness: 30 mm) and frozen at −80°C for five to six hours. The ice samples were irradiated by UVC-LED or conventional UV lamp at −30°C. Following UVC treatment, the samples were melted at room temperature, and appropriate serial dilutions (1/10) were performed using sterile 0.1% peptone water. Dilutions were plated on duplicate tryptic soy agar plates, and then incubated at 37°C for 24 to 48 h.

Results: The UVC-LED inactivated the pathogenic bacteria in ice more efficiently than the conventional UV lamp. Pathogens contained in ice at a concentration of 10^6 to 10^7 CFU/ml were inactivated by UVC-LED at a UV dosage of 160 mJ/cm^2, regardless the species of pathogen. UVC-LED irradiation from two different directions more efficiently inactivated bacteria than two irradiation cycles from the same direction.

Significance: The results presented here suggest that UVC-LED could contribute to ensuring the microbiological safety of ice.

T9-09 Effect of Gaseous Ozone on Foodborne Pathogens and Their Surrogates on Fresh and Frozen Strawberries

ZIJIN ZHOU[1], Frédérique Cantergiani[2], Frank Devlieghere[1], Sophie Zuber[2] and Mieke Uyttendaele[1]
[1]Ghent University, Ghent, Belgium, [2]Nestlé Research Center, Lausanne, Switzerland

❖ ***Developing Scientist Competitor***

Introduction: Ozone technology has been widely applied in water disinfection and is used in food industry to extend the shelf life of fresh produce with well-documented bactericidal effects. With the increasing number of outbreaks related to berry products, alternative technologies need to be investigated to improve the safety of berries. However, information on the application of gaseous ozone on fresh produce is limited and not comparable due to various expressions of ozone dose.

Purpose: Our study investigated the inactivation effect of gaseous ozone on foodborne pathogens on fresh and frozen strawberries and the sensorial impact of gaseous ozone on strawberries.

Methods: Fresh strawberries (25 g) were inoculated with MS2, MNV-1, *Salmonella,* and *E. faecium* and treated with gaseous ozone at 1% (ca. 15g/m^3) and 6% (ca. 80g/m^3) for five and 30 minutes or pure oxygen for 40 minutes. Frozen strawberries were treated with 6% ozone for five minutes. After treatment, samples were transferred into a filter-stomacher bag containing either 50 ml virus elution buffer or 225 ml buffered peptone water to recover and enumerate viruses and bacteria, respectively. All experiments were done in triplicates.

Results: For frozen strawberries, treatment with ozone resulted in log reductions of 1.60, 0.72, 0.67, and 1.77 on *Salmonella*, *E. faecium*, MNV-1, and MS2, respectively, which were higher compared to fresh samples treated in parallel. Treatments of 6% for 30 min provided the highest reduction on fresh strawberries; 2.06-, 1.52-, 1.76-, and 3.30-log reductions were reached on *Salmonella*, *E. faecium*, MNV-1, and MS2, respectively, which were significantly higher than oxygen treated samples (P<0.05). No sensorial differences were detected on strawberry flesh but green leaves were decolorized by ozone.

Significance: This study proved the inactivation effect of gaseous ozone against pathogens without altering the taste of strawberries and showed more efficiency on the frozen strawberry matrix compared to fresh strawberries.

T9-10 Thermal Process for Inactivating *Listeria monocytogenes* on Surfaces of Whole Fresh Cantaloupes

BASSAM ANNOUS[1] and Angela Burke[2]
[1]U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, [2]U.S. Department of Agriculture-ARS, Wyndmoor, PA

Introduction: Outbreaks of listeriosis by *Listeria monocytogenes* have been associated with the consumption of cantaloupes. Commercial washing processes for cantaloupes are limited in their ability to inactivate and/or remove this human pathogen.

Purpose: Our objective was to develop a surface pasteurization process for enhancing microbiological safety of cantaloupes.

Methods: Whole cantaloupes, surface inoculated with three strains of *L. monocytogenes* were stored at 4°C for 24 h prior to processing. Cantaloupes were, then, treated at 80°C for 210 s. Fresh-cut samples were prepared from treated and non-treated cantaloupes and stored at 6°C for up to 12 days.

Results: Hot water treatments (80°C for 210 s) reduced *L. monocytogenes* populations in excess of 5 log CFU/g rind. Cantaloupes that were treated and stored at 6°C for 12 days retained their firmness qualities and maintained nondetectable levels of *L. monocytogenes* as compared to the controls. Also, levels of *L. monocytogenes* on fresh-cut cantaloupes, prepared from treated cantaloupes and stored for 12 days at 6°C, were nondetectable as compared to the controls.

Significance: These results indicate that the hot water surface pasteurization at 80°C for 210 s will enhance the microbiological safety of fresh and fresh-cut cantaloupes and will extend the shelf life of this commodity. These results provide a framework to packers/producers of fresh and fresh-cut cantaloupes for the potential use of hot water as an intervention treatment for enhancing the microbiological safety and qualities of this commodity.

T9-11 Inactivation of *Salmonella* in Corn Flour by Radio Frequency Heating and the Effect of Cold Shock

SAMET OZTURK[1], Fanbin Kong[1], Rakesh K. Singh[1], Juming Tang[2] and Shuxiang Liu[2]
[1]University of Georgia, Athens, GA, [2]Washington State University, Pullman, WA

❖ ***Developing Scientist Competitor***

Introduction: Radio Frequency (RF) heating has shown a potential to control pathogens in low moisture foods.

Purpose: The study aimed to: i) validate RF heating treatment to inactivate *Salmonella* Enteritidis in corn flour, as a model low moisture food, and ii) study the effect of holding and cold shock in enhancing RF inactivation.

Methods: Corn flour (moisture content 10.8%) was inoculated with *Salmonella* Enteritidis PT30 (*Salmonella* PT30) at approximately 10^8 CFU/g. Inoculated samples (0.7 g) were heated in a water bath at 75, 80, and 85°C for predetermined time periods to determine *D* and *z*-values. Corn flour (seven pounds) was loaded into a polyetherimide (PEI) container (7 by 24 by 30 cm^3) with prepackaged inoculated corn flour (five grams) placed in the geometric center. The samples were subjected to RF heating to reach temperatures of 75, 80, and 85°C, respectively, and held for 10 min after heating. The *Salmonella* PT30 survivors in heated samples with/without holding were enumerated. To study the effect of cold shock, inoculated samples (20 g) were loaded into small plastic bottles (height 6.3 by diameter 3.4 cm), which were subjected to RF heating to reach 80 and 85°C. The heated samples were kept in a freezer at -20°C for 48 and 96 h before the *Salmonella* survivors were evaluated.

Results: The *D*-values of *Salmonella* PT30 at 75, 80, and 85°C were 14.2, 6.1, and 2.03 min, respectively, and the *z*-value was 11.9°C. Heating only (without holding) to reach 75, 80, and 85°C temperatures had caused 0.9-, 2.02-, and 2.83-log reductions of *Salmonella* in the corn flour, respectively. Holding samples for 10 min in the container further resulted in 0.45 to 1.53 log-reduction. Freezing of the heated samples for 96 h caused around 0.6 to 1.8 log reduction.

Significance: Results demonstrated that RF heating treatments effectively inactivated *Salmonella* PT30 in packaged corn flour and that RF combined with holding and cold shock significantly enhanced the inactivation effect.

T9-12 Inactivation of *Listeria monocytogenes* on the Surface of Smoked Salmon by Riboflavin-based, 460nm Light Emitting Diode Illumination

Min-Jeong Kim[1], Lianto Dian Kartikasari[1] and **HYUN-GYUN YUK**[2]

[1]National University of Singapore, Singapore, Singapore, [2]Korea National University of Transportation, Chungju-si, South Korea

Introduction: It is known that the antimicrobial effect of blue light emitting diodes (LED) could be enhanced by the addition of photosensitizers, such as riboflavin. However, little information is available on the effectiveness of blue LED with the addition of photosensitizers in eliminating food-borne pathogens on ready-to-eat fish products.

Purpose: The aim of this study was to compare the bactericidal efficacies of 460nm LED illumination, with various photosensitizers, against *Listeria monocytogenes* in phosphate buffered saline (PBS) and to evaluate the effectiveness of riboflavin-based LED illumination on the inactivation of *L. monocytogenes* on the surface of smoked salmon at chilling temperatures.

Methods: A bacterial suspension (10^7 CFU/ml) in PBS containing each photosensitizer [5-aminolevulinic acid (ALA), chlorogenic acid (CA), and riboflavin (RF)] was illuminated with 460nm LED at 20°C for 30 min (0.2 kJ/cm^2) to find the most effective photosensitizer. For food application, the smoked salmon inoculated with *L. monocytogenes* cocktail culture (10^4 CFU/cm^2) was treated with RF and then illuminated by LED for 48 h (12.9 kJ/cm^2) at 4 and 10°C.

Results: A 30 min treatment with LED illumination, alone, reduced L. monoctyogenes in PBS 1.5 log CFU/ml, while illumination with ALA, CA, and RF inactivated *L. monocytogenes* by 2.8, 2.8, and 4.2 log CFU/ml, respectively; revealing that RF was the most effective photosensitizer. On the surface of smoked salmon, LED illumination (48 h at 4°C) with and without RF decreased 0.7 and 0.4 log CFU/cm^2 of *L. monocytogenes* populations, respectively. At 10°C, bacterial populations on non-illuminated and illuminated samples increased up to 5.7 and 4.4 log CFU/cm^2, respectively; no growth was observed in LED-illuminated salmon with RF for 48 h.

Significance: These results suggest that riboflavin-based 460nm LEDs could be a potential technology to preserve smoked salmon at chilling temperatures, minimizing the risk of listeriosis.

T10-01 A Quantitative Risk Model to Assess Post-harvest Parameters that Impact the Levels of *Salmonella* on Pistachios

JAVAD BAROUEI[1], Donald W. Schaffner[2] and Linda J. Harris[3]

[1]University of California-Davis, Department of Food Science and Technology, Davis, CA, [2]Rutgers University, Department of Food Science, New Brunswick, NJ, [3]University of California-Davis, Davis, CA

Introduction: Pistachios are harvested at >40% moisture, hulled, separated into floater and sinker streams, dried to <7% moisture, and stored in large silos. In a recent survey, the prevalence of *Salmonella* was ~0.4% and ~2% in raw dried sinkers and floaters, respectively; mean contamination levels of 0.18 and 0.66 MPN/100 g were observed in positive samples.

Purpose: The current study develops a Monte Carlo-based simulation model to identify postharvest parameters may influence the prevalence and levels of *Salmonella* in floater and sinker pistachios.

Methods: Time estimates for transport from the orchard to the hulling facility (transportation delay) and for posthulling holding before drying (drying delay) were derived from industry data. The lag, log and maximum population changes of *Salmonella* in inoculated inhull and hulled floater and sinker pistachios were determined in laboratory studies. Estimated reductions of *Salmonella* during pistachio drying were also analyzed and translated to appropriate distributions to build the model. Initial contamination was assumed to be confined to 100 g in a 25,000 kg truckload with a localized (limited to 1,000 g) contamination pattern. The model was used to predict final levels of *Salmonella* in 100,000 truckloads of harvested pistachios.

Results: Simulation results using initial levels of 0.2 CFU/100 g and assuming localized distribution closely matched survey outcomes. Significantly more *Salmonella* were predicted on floaters (mean 0.62 MPN/100 g, 95% CI, 0.00017 to 100,000 MPN/100 g) than on sinkers (mean 0.16 MPN/100 g, 95% CI, 0.00013 to 10,000 MPN/100 g). Transportation delays significantly impacted the model outcomes [correlation coefficient (CC) 0.97 and 0.87 for sinkers and floaters, respectively] but drying delays were more strongly correlated with final *Salmonella* levels in floaters (CC 0.40) than sinkers (CC 0.11). Predicted *Salmonella* levels decreased significantly by reducing delays in transportation.

Significance: This model has potential as a pistachio harvest management tool to enhance the microbial safety of pistachios.

T10-02 Farm to Fork Quantitative Microbial Risk Assessment for Norovirus on Frozen Berries

ROBYN MIRANDA[1] and Donald W. Schaffner[2]

[1]Rutgers University, New Brunswick, NJ, [2]Rutgers University, Department of Food Science, New Brunswick, NJ

❖ ***Developing Scientist Competitor***

Introduction: Frozen fruit has been linked to Norovirus outbreaks throughout the world. Contamination sources on the farm have been attributed to the use of contaminated irrigation water and infected food handlers. A quantitative microbial risk assessment describing Norovirus prevalence and concentration during preharvest, harvest, postharvest processing, storage, preparation, and consumption of berries is presented.

Purpose: The purpose of this QMRA was to simulate the prevalence and concentration of Norovirus on frozen berries from preharvest through distribution, preparation, and consumption. The output of the model is predicted number of illnesses. The model simulated the largest known outbreak arising from Norovirus-contaminated berries. The outbreak occurred in 2012, in Germany, and was linked to frozen strawberries sourced from China.

Methods: The model was built in Excel using the Monte Carlo modeling software @Risk add-in. The QMRA was composed of five modules: in field, processing and packaging, storage, preparation, and consumption. Data from the scientific literature regarding Norovirus behavior in fresh and frozen fruit were used to develop the model.

Results: The literature data indicated transfer of Norovirus from hands to berries can be as great as 60±43%, if the berry is wet, and as low as 0.10±0.04%, when the berry is dry. Postharvest washing of berries with chlorinated water (1.7±0.4 mean log reduction) was significantly more effective than washing with warm (0.8±0.2 mean log reduction) or cold water (0.6±0.3 mean log reduction). Freezing and frozen storage results in a log reduction from 0.2 to 1.1 depending on freezing conditions and frozen storage time.

Significance: The model was successfully able to simulate the German frozen strawberry outbreak. Scenario analysis showed that heating the frozen berries prior to consumption was the most effective means to prevent illness.

T10-03 A Farm to Fork Exposure Model Predicting *Alternaria* Mycotoxin Exposure from Derived Tomato Products Evaluating Impact of Climate Change and Processing Conditions

LIESBETH JACXSENS, Bruno De Meulenaer and Frank Devlieghere

Ghent University, Ghent, Belgium

Introduction: A probabilistic *Alternaria* mycotoxin farm to fork exposure model was developed describing pre-harvest mold growth and potential production of mycotoxins alternariol (AOH) and alternariol monomethylether (AME) on ripening tomatoes in production fields in Spain and Poland. After harvesting, the production process of tomato products is exemplified by tomato concentrate.

Purpose: The outcome of the model gives the distribution of exposure to AOH and AME due to the consumption of derived tomato products for the Belgian population, exemplifying the European consumers, and its relation to different climate change scenarios.

Methods: A full risk assessment research was conducted and @Risk Software was applied to perform Monte Carlo simulations.

Results: The hot break production process resulted in a lower exposure compared with the cold break procedure due to the effect of the heating on the stability of the mycotoxins. The calculated mean exposure for AOH was 0.004 µg/kg BW.day residing in the range reported by EFSA between 0.0036 to 0.026 µg/kg BW.day (lower bound-upper bound; EFSA, 2011). For AME, a higher mean exposure was calculated compared to AOH, being 0.008 µg/kg BW.day and following EFSA calculations of 0.01 to 0.063 µg/kg BW.day (lower bound-upper bound). Several climate change scenarios were evaluated that impact the pre-harvest mold growth and potential mycotoxin production. These show that in extreme RPC scenarios (6.0 and 8.5 in Spain), which result in an increase in temperature (18.2 to 38.2°C), the exposure will be lower due to temperatures that are too high for the growth of *Alternaria* molds. Simulations also demonstrated that Poland will shift in the far future (2081 to 2100) to the present situation of Spain (1981 to 2000) with temperatures of 14.1 to 28.4°C.

Significance: This paper emphasizes the importance of preventive measures at fields to avoid the prevalence of *Alternaria* molds and sorting the moulded tomatoes out before entering the production line of derived tomato products.

T10-04 Evaluating the Relative Impact of Swine Deep Tissue Lymph Nodes on Human Salmonellosis Due to Consumption of Ground Pork Based on Quantitative Simulation Modeling

YANGJUNNA ZHANG[1], Annette O'Connor[2], Chong Wang[2], James Dickson[2] and Bing Wang[1]

[1]University of Nebraska-Lincoln, Lincoln, NE, [2]Iowa State University, Ames, IA

Introduction: The consumption of pork products is considered one of the major causes for human salmonellosis throughout the world, among which wholesale fresh ground pork (WFGP) has drawn increasing attention. The inclusion of deep tissue or nonvisceral lymph nodes (DTLNs) contaminated with *Salmonella*, probably occurring during WFGP processing, may pose risks to public health.

Purpose: This study assessed the relative contribution of DTLNs to human salmonellosis in the United States and investigated the critical control points for reducing the risk.

Methods: A stochastic simulation model was established to quantify the level of risk.

Results: The model predicted an average of 170 cases of salmonellosis with 95% confidence interval from 117 to 223 per 100,000 consumers, annually, due to ground pork consumption. The scenario analysis showed interventions relating DTLNs, such as complete removal of DTLNs during processing, could be associated with a slight reduction in the average number of *Salmonella* in WFGP (7.4 to 6.3 CFU/serving), but a significant decrease in the prevalence of *Salmonella*.

Significance: These observations indicate the removal of DTLNs at processing plants might not be as effective as other interventions in reducing the risk.

T10-05 Prioritizing Pig Farms in the Netherlands to Reduce the Foodborne Disease Burden of *Toxoplasma gondii*

MARTIJN BOUWKNEGT, Derk Oorburg and Bert Urlings

Vion, Boxtel, Netherlands

Introduction: Toxoplasma gondii is a priority foodborne pathogen in the Netherlands, estimated to cause a foodborne health loss of approximately 2,000 DALY and an economic loss of M€ 11, annually. Estimates for the contribution of pork to the human disease burden in the Netherlands range from 10 to 50% in different studies.

Purpose: This study aimed to identify pig farms that contribute most to the pork-related foodborne toxoplasma disease burden, as prime candidates for intervention. The contribution is estimated from the true infection status at animal-level per farm, the likelihood of the presence of tissue cysts given the serological result, and the number of pigs delivered to slaughter.

Methods: The true infection status was estimated from *T. gondii* antibodies in serum using a general Bayesian latent class hierarchical model. From each pig herd delivered to three Dutch slaughterhouses in 2012 to 2016, one to six blood samples were collected and examined serologically with the PrioCHECK™ Toxoplasma Antibody ELISA Kit (Thermo Fisher). The likelihood of tissue cysts for serologically positive pigs was quantified from literature data. The number of pigs delivered per farm were obtained from slaughterhouse records. Sensitivity analyses were conducted to assess the effect of prior choice in the latent class model.

Results: In total, 226,839 samples of 174,583 herds delivered from 3,521 farms were tested serologically. Preliminary analyses gave estimates for diagnostic test sensitivity and specificity of approximately 80% (95% credible interval: 42 to 99%) and 99.3% (99.1 to 99.5%), respectively. Estimates for the herd-level prevalence, animal-level prevalence, and the contribution to the disease burden per pig farm are currently being finalized.

Significance: Prioritizing pig farms according to their relative contribution to the foodborne disease burden supports the highest health gain for intervention programs. This study produced a robust methodology for that purpose and showed its application in the Netherlands for the priority foodborne pathogen, *T. gondii*.

T10-06 Developing a Risk Management Framework to Improve Public Health Outcomes by Enumerating *Salmonella* in Ground Turkey Products

FERNANDO SAMPEDRO[1], Scott Wells[1], Jeff Bender[1] and Craig Hedberg[2]

[1]University of Minnesota, College of Veterinary Medicine, St. Paul, MN, [2]University of Minnesota, School of Public Health, Minneapolis, MN

Introduction: Rates of *Salmonella* infection in the United States have averaged 15.2 cases per 100,000 population (range, 13.6 to 16.4) from 1996 to 2015. Performance standards based on prevalence of *Salmonella* in raw meat and poultry have reduced prevalence of contamination, but not prevented outbreaks of foodborne disease.

Purpose: This study evaluated the potential to improve public health outcomes (reduction of the number of *Salmonella*-related illnesses and outbreaks) by enumerating *Salmonella* in ground turkey products with the goal of focusing control measures on levels of contamination associated with outbreaks.

Methods: A risk assessment model was developed to estimate the national occurrence of *Salmonella* infections attributable to ground turkey, based on current human salmonellosis incidence, data on prevalence, and enumeration of *Salmonella* in ground turkey products, patterns of ground turkey consumption, and dose-response models for infection. Model parameters were estimated from published data. The impact of removing product contaminated at one colony forming unit (CFU) of *Salmonella* per gram of ground product from the fresh and frozen distribution chain was evaluated. Outbreak-specific attack rates associated with FSIS recall data were used to validate model estimates.

Results: The model estimated a median of 1,301 *Salmonella* infections per year. This would result in 45 *Salmonella* cases reported to public health officials, with an upper bound exceeding 4,000 cases. The net effect of removing lots with > one CFU/g contamination was a 21% reduction in median cases and an 82% reduction in the estimated upper bound of reported cases. At this threshold level of contamination, only two *Salmonella* infections and no reported cases would be expected from distribution of a single 2,000 pound lot of contaminated product. Distribution of at least 70 such lots would be required to produce a detectable cluster of reported cases.

Significance: The model demonstrated the relationship between levels of *Salmonella* in ground turkey products and public health outcomes, including the recognition of potential foodborne outbreaks.

T10-07 Farm-to-Fork Risk Assessment of *Listeria monocytogenes* in Cold-smoked Salmon in Scotland

STOYKA CHIPCHAKOVA, Francisco Perez-Reche, Kenneth Forbes, Ovidiu Rotariu, David Watts and Norval Strachan

University of Aberdeen, Aberdeen, United Kingdom

❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* can contaminate cold-smoked salmon from various sources and can persist through processing. However, existing risk assessment models of *L. monocytogenes* in cold-smoked salmon focus only on the consumption stage.

Purpose: This research aims to determine the critical control points (CCPs) for *L. monocytogenes* contamination along the production chain in the Scottish cold-smoked salmon industry and to estimate the probability of illness in consumers.

Methods: An Excel spreadsheet model was built with variables accounting for: processing and storage conditions along the food chain, prevalence, demographics and consumption data. Monte Carlo simulations and sensitivity analysis were performed using @Risk. The probability of illness per serving was estimated through an exponential dose-response model.

Results: The prevalence of *Listeria* in cold-smoked salmon was most sensitive to cross-contamination during transport to smoking plant (Spearman Rank coefficient 0.87), contamination during slicing (0.87), filleting (0.86), and salting (0.84). The dose-response model was most sensitive to *Listeria* prevalence in the final product (0.78) and *Listeria* contamination levels (0.50). The model used a species specific constant R (1.18×10^{-10}) that helps define the dose-response curve. The estimated mean risk of illness per serving was 6.41×10^{-06}. Assuming 100% virulence of *L. monocytogenes* strains, the average number of predicted listeriosis cases in Scotland was 49, whereas the reported annual average is 17.

Significance: This farm-to-fork risk assessment of *L. monocytogenes* in cold-smoked salmon suggests that CCPs are transport to smoking plant, slicing, filleting and salting. Appropriate controls in these stages would improve food safety through reduction of *Listeria* prevalence and levels in cold-smoked salmon.

T10-08 Assessment of Public Health Risk Associated with Formalin Exposure in Fish in Southern Bangladesh

Md. Sazedul Hoque[1], **LIESBETH JACXSENS**[2], Md. Boktheir Hossain[1], SM Oasiqul Azad[1], AKM Nowsad Alam[3], Bruno De Meulenaer[2], Carl Lachat[2] and Md. Shahin Alom[1]

[1]Patuakhali Science and Technology University, Department of Fisheries Technology, Faculty of Fisheries, Patuakhali, Bangladesh, [2]Ghent University, Ghent, Belgium, [3]Bangladesh Agricultural University, Department of Fisheries Technology, Faculty of Fisheries, Mymensingh, Bangladesh

Introduction: Formalin is reported to be frequently added as a preservative to fresh fish to prevent spoilage and extend shelf life. The carcinogenicity and mutaginicity of formalin is a challenging public health issue in Bangladesh.

Purpose: This study assessed the public health risk associated with formalin exposure in fresh water fish in Southern Bangladesh.

Methods: Formalin concentrations (mg/kg) were determined (n=3) in tilapia, Indian major carp rui, Chinese carp, and a minor carp from the local market and in laboratory simulations (0.5, 1.0, 2.0, and 4.0% formalin solution for 5, 15, 30, and 60 min) with spectrophotometric and HPLC method. A food frequency questionnaire was used to collect fish consumption (kg/kgBW.day) data from 400 respondents. A probabilistic exposure assessment was conducted using @Risk®7.0 software.

Results: Fish treated with increasing concentrations of formalin and time showed increased trends of formalin acquisition, irrespective to fish species and analytical methods used (P<0.05). The questionnaire survey showed higher consumption of rui and tilapia than Chinese carp and minor carp. Under spectrophotometric analysis, formalin exposure with consumption of four different fish was lower than ADI (0.2 mg/kgBW.day) and TDI (0.15 mg/kgBW.day) value for both total population (400 respondents, both consumer and nonconsumers) and consumers. Maximum exposure of formalin (0.28 mg/kg BW.day) was for tilapia (consumers) determined by the HPLC method. MoE provides high priority (<10,000) for tilapia and rui at P99 under spectrophotometric analysis; whereas, under HPLC analysis, tilapia had much lower MoE values at P99, P95, and P90 (total population and consumers).

Significance: This study suggested that exposure to formalin associated with tilapia consumption is a public health concern in Southern Bangladesh. Thus, it is a priority for risk management strategies and should be taken into consideration.

T10-09 Performance Assessment of the Canadian Food Inspection Agency Establishment-based Risk Assessment Model

Cecile Ferrouillet[1], Manon Racicot[2], Alexandre Leroux[3], Mathieu Cormier[4], **ROMINA ZANABRIA**[5], Julie Arsenault[1], Ann Letellier[1], Anna Mackay[3], Ashwani Tiwari[3], Solomon Aklilu[3], Mansel Griffiths[6], Richard Holley[7], Tom Gill[8], Sylvain Charlebois[8] and Sylvain Quessy[9]
[1]University of Montreal, St-Hyacinthe, QC, Canada, [2]Canadian Food Inspection Agency, St-Hyacinthe, QC, Canada, [3]Canadian Food Inspection Agency, Ottawa, ON, Canada, [4]Canadian Food Inspection Agency, Montreal, QC, Canada, [5]Canadian Food Inspection Agency, Guelph, ON, Canada, [6]University of Guelph, Guelph, ON, Canada, [7]University of Manitoba, Winnipeg, MB, Canada, [8]Dalhousie University, Halifax, NS, Canada, [9]University of Montreal, Saint-Hyacinthe, QC, Canada

Introduction: The Canadian Food Inspection Agency (CFIA) is modernizing its risk-based approach to oversight by developing the establishment-based risk assessment model to enhance a more effective and efficient allocation of inspection activities to highest risk areas. Its initial developmental stages involved the selection/refinement of key risk factors, the identification of assessment criteria, and individual weighting.

Purpose: This study was undertaken to assess the performance of the model.

Methods: A pilot project was conducted involving 80 meat/poultry and dairy establishments that were selected based on a nonproportional, stratified, random sampling and 65 CFIA senior inspectors with a minimum of five years of experience in food safety. Each expert categorized 10 randomly selected establishments including two controls, based on their risk to human health, using an ordinal scale that was later translated to interval variables. Performance evaluation occurred by correlating the model outputs (estimated as the annual number of disability adjusted life years) with the ranking scores given by senior inspectors to the same pilot establishments. This step also aimed at refining the model by identifying specific criteria responsible for discrepancies between both assessments.

Results: Data analysis showed the Spearman correlation coefficient to be highly positive for large meat/poultry and medium dairy size establishments (r=0.73, *P*=0.01 and r=0.89, *P*<0.001, respectively) and moderately positive for meat/poultry storage and dairy processors of moderate risk products establishments (r=0.69, *P*=0.01 and r=0.62, *P*<0.01, respectively). Few discrepancies were observed when considering the facility type, volume/type of product, and the inspection compliance results. Although experts might have considered differently the impact of specific factors when assessing establishments compared to the model, the criteria responsible for this disagreement were not identified after using the Kruskal-Wallis test.

Significance: Pilots with other food commodities are currently in progress and will further help enhance the CFIA risk assessment model that will be implemented as part of the regulatory oversight activities of the Agency.

T10-10 Bayesian Approach to the Evaluation of the Potential Impact of Climatic Change on Hepatocellular Carcinoma Risk Attributable to Chronic Aflatoxins Exposure through Food

PATRICK NJAGE and Joseph Wambui
University of Nairobi, Nairobi, Kenya

Introduction: Epidemiological studies show a definite connection between aflatoxin exposure and a high occurrence of human hepatocellular carcinoma (HCC). At the same time, changes in climatic conditions may impact the growth of Aspergillus flavus and production of toxins. Nevertheless, little is known about the actual impact of climatic change on changes in the risk of food associated HCC. This risk, therefore, receives scant attention despite evidence of a rapid increase in cases of HCC.

Purpose: We present projections on changes in climatic patterns and the associated complex and dynamic changes in aflatoxin contamination in different regions of Kenya, where the problem of aflatoxin contamination in cereal crops is endemic.

Methods: The risk attributable to consumption of contaminated maize and groundnuts was assessed. Bayesian statistics provided a unified probabilistic approach for handling uncertainty and variability. The enhancement of the risk by prior hepatitis B virus diagnosis in aflatoxin exposed individuals was, also, assessed.

Results: Contaminated maize and groundnuts contributed additively to the risk of aflatoxin associated HCC cases of greater than 2,000 cases per annum. Changes in climatic patterns (rains and temperatures) enhance this risk, differently, from region to region. Regions previously associated with lower aflatoxin contamination levels such as central and western regions will experience an increase in risk. However, decreased HCC risk is projected in eastern and southern regions regions, which currently experience higher cases of aflatoxicosis. Several management practices including agronomic, postharvest, and biologic measures show high potential in mitigating the risk, especially when applied in combination.

Significance: Climatic changes will, albeit differently, enhance foodborne HCC risk. Both the risk and potential mitigation measures are suggested.

T10-11 Development of a Probability Model to Describe the Variability in the Time to Inactivation of *Salmonella enterica*

KENTO KOYAMA, Hidekazu Hokunan, Mayumi Hasegawa, Shuso Kawamura and Shigenobu Koseki
Hokkaido University, Sapporo, Japan

❖ ***Developing Scientist Competitor***

Introduction: Despite the development of many predictive models, variability in time to inactivation of bacterial populations still cannot be precisely estimated. It is difficult to evaluate the risk of surviving bacteria in foods after inactivation treatment, although a few remaining bacterial cells, such as *Salmonella enterica* or enterohaemorrhagic *Escherichia coli*, can cause foodborne infection. Accurate estimation of death probability for a bacterial population would be useful for determining the risk of the pathogenic bacteria. This is particularly important in low water activity foods.

Purpose: This study was undertaken to develop a probability model to estimate the death probability of a *S. enterica* Typhimurium population under conditions of desiccation over time.

Methods: Bacterial cells were prepared with an initial concentration adjusted to 1×10^{n} CFU/µl (where *n*=1, 2, 3, 4, 5) by 10-fold dilution. The inocula was dispensed into 96-well microplates (2 µl per well). The microplates were stored under desiccating conditions (10 to 20% relative humidity) at 5, 15, or 25°C. The survival of bacterial cells in each well was assessed by adding 100 µl of tryptic soy broth as an enrichment culture at arbitrary time intervals.

Results: The changes in the death probability of 96 replicate bacterial populations were described as cumulative gamma distribution. Variability in time to inactivation was described to transform a cumulative gamma distribution into a gamma distribution. In addition, the certainty levels were described for bacterial inactivation that ensure death probability of a bacterial population at six different levels, from 90 to 99.9999%.

Significance: The study results supported the use of the model for describing death probability of a bacterial population. Death probability is useful in risk assessments that estimate bacteria remaining after inactivation processes.

T10-12 Estimating Risk Attributed to Food-handling Behaviors in Retail and Households

IOANA (JULIA)

MARASTEANU[1], Girvin Liggans[2], Jessica Otto[2] and Angela Lasher[2]

[1]U.S. Food and Drug Administration, Silver Spring, MD, [2]U.S. Food and Drug Administration, College Park, MD

Introduction: The prevention of foodborne illness remains a significant priority to public health agencies in the United States. Given the prevalence of multiple contributing factors that can lead to foodborne illness at both the retail and household levels, it is important to understand how individual food handling practices combine to impact the incidence of foodborne illness.

Purpose: The purpose of this study was to introduce the Food Handling Practices Model (FHPM), its assumptions, and applications.

Methods: The FHPM is a quantitative model that tracks servings of food (1,127,245,021,184 annually) as they go through various stages leading to noticeable foodborne illness. The model consists of 1,546 parameters and assumes interlinked binomial random variables. It runs Monte Carlo simulations based on inputted parameters, and estimates the number foodborne illnesses, hospitalizations, and deaths associated with the specified parameters. Baseline and change scenarios are compared to assess the change in risk due to changes in food handling practices.

Results: Among many change scenarios presented, the top five contributing factors leading to foodborne illness at the retail level (household level) are: inappropriate behavior related to cooling (room temperature holding), thawing (thawing), cold holding (raw or lightly cooked food), advance preparation (cooking), and hot holding (cooling); with approximately 25,382 (268,093), 24,180 (212,115), 23,400 (208,241), 20,330 (194,313), and 19,771 (171,812) illnesses avoided annually per one percent reduction in incidence, respectively. Change scenario results can be inferred from each other because the model is linear and the results of most change scenarios are independent.

Significance: The FHPM is an important tool for analyzing risk associated with food handling practices at the retail and household level and can inform policy makers' decisions relating to food safety interventions.

T11-01 Effect of Moisture, pH, and Salt on Pathogen Lethality and Stabilization in Ham with Extended Come-up and Extended Cooling Profiles

MAX GOLDEN, Amanda Skarlupka, Katie Osterbauer, Jeffrey Sindelar and Kathleen Glass

University of Wisconsin-Madison, Madison, WI

Introduction: USDA-FSIS Appendices A and B are widely used as support for thermal process validation and cooling. Bacterial growth in cured ham during extended dwell times, prior to or immediately after lethality, have not been investigated.

Purpose: This study was conducted to determine the effect of pH, moisture, and salt in combination with extended cook and cool on the growth/survival of pathogens in cured ham.

Methods: Ten cured ham (156 mg/kg $NaNO_2$) treatments were formulated to represent ranges of 55 to 75% moisture, pH 5.8 to 6.4, and 1.5 to 3.0% salt using a full factorial design. Raw treatments were inoculated with three log CFU/g *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp., or *Clostridium perfringens* spores and vacuum packaged (25 g/package). Samples were heated (linear increase from 10 to 54°C) according to Appendix A (in 6 hours) or slow-cook (in 10 hours), and then continued heating to an internal temperature of 70°C. Samples were slow cooled from 54 to 4°C in 25 hours (extended from Appendix B). Duplicate samples per treatment were assayed at zero-time and at internal temperatures 32, 54, 70, 29, and 4°C by enumerating on appropriate selective agars.

Results: All treatments inoculated with *L. monocytogenes, S. aureus, Salmonella* spp., or *C. perfringens* inhibited growth during control and slow cook. All three vegetative pathogens were inactivated by cooking to 70°C. No difference in survival was observed between Appendix A and slow-cook treatments. In contrast, *C. perfringens* increased 4.7, 3.0, and 1.7 log during the 25-h extended cooling in 75% moisture treatments with pH 6.4/1.5% NaCl, pH 6.4/3.0% NaCl, and pH 5.8/1.5% NaCl, respectively. In addition, 55% moisture ham with pH 6.4 and 6.1 and low salt supported a >1.5 log increase. None of the other treatments supported growth during the 25-h extended cool.

Significance: This study confirmed the critical nature of salt, pH, and moisture for pathogen inhibition in cured meat during extended dwell times.

T11-02 Tuning the Bloodhound® VOC Analyzer to Detect *Campylobacter* during Broiler Poultry Production

LYNN MCINTYRE[1] and Tim Gibson[2]

[1]Harper Adams University, Newport, United Kingdom, [2]RoboScientific Ltd, Leeds, United Kingdom

Introduction: The broiler commensal *Campylobacter* is estimated to cause >280,000 human disease cases annually in the United Kingdom via consumption of undercooked contaminated meat or through cross-contamination during food preparation. Detecting the presence of *Campylobacter* in broiler flocks preslaughter is one of many approaches suggested to reduce its prevalence on processed broiler meat, but industry adoption of farm-level testing has been limited due to the absence of a real-time, on-site monitoring method.

Purpose: This study optimized the detection of *Campylobacter* spp. by the Bloodhound®VOC Analyzer based on volatile organic compounds (VOCs) produced.

Methods: Thirteen *Campylobacter* (*Campylobacter jejuni, Campylobacter coli, Campylobacter lari, and Campylobacter upsaliensis*) and 12 non*Campylobacter* isolates (including *Salmonella* spp., *Listeria monocytogenes*, *Arcobacter* spp. and *Bacteriodes fragilis*) were cultured on CBA at 37°C for 24 to 48 h under appropriate atmospheric gas conditions. Culture plates were heat sealed in polyethylene sachets inflated with clean air. Lids were removed allowing the VOCs to equilibrate in the headspace before sampling (*n*=12 per isolate). This was repeated following sensor tuning. Linear Discriminant Analysis was used to process the raw data.

Results: Tuning of the Bloodhound® sensors improved the detection of *Campylobacter* spp. within a shorter time period. Prior to sensor tuning, all 48 h old *Campylobacter* spp. cultures were successfully detected and differentiated from control and nontarget culture plates with Mahalanobis distances ranging from 405 to 652. Sensor tuning increased the Mahalanobis distances to between 13,510 and 17,391. This improvement enabled differentiation of *Campylobacter* spp. Sensor tuning also effected earlier detection of *C. jejuni* by increasing the sensitivity of the sensors 21 times for the 24 h culture and 158 times for the 48 h culture.

Significance: Applying tuned sensors to an on-farm monitoring system will improve the speed and reliability of *Campylobacter* detection, which will promote better control via flock management.

T11-03 Antimicrobial Neutralization Ability of Buffered Peptone Water Compared to Neutralizing Buffered Peptone Water on *Salmonella*-inoculated Broiler Carcasses

JENNIFER VUIA-RISER, Christine Alvarado, Christopher Kerth and Matt Taylor
Texas A&M University, College Station, TX

❖ ***Developing Scientist Competitor***

Introduction: The United States Department of Agriculture (USDA) recently amended the protocol for poultry testing to utilize neutralizing buffered peptone water (nBPW) as the rinse fluid for broiler carcass pathogen testing in an effort to improve the capacity for pathogen detection during routine testing. Application of antimicrobial interventions during poultry harvest may interfere with the detection of microbial pathogens due to carryover of antimicrobials into carcass rinse solutions.

Purpose: The purpose of this study was to compare the antimicrobial neutralizing ability of BPW versus nBPW on broiler carcasses inoculated with a mixture of antibiotic-resistant *Salmonella enterica* serovars.

Methods: Young broiler carcasses were collected and inoculated with a *Salmonella* inoculum containing serovars Typhimurium, Enteritidis, and Heidelberg, resistant to 100.0 µg/ml rifampicin, at 6.0±0.4 log CFU/ml. Inoculated carcasses were treated by peracetic acid (PAA; 0.2%) or cetylpyridinium chloride (CPC; 0.8%) rinse, or a CPC rinse followed by 80 min ice-water bath (CPC_{80}; 0.8%) (*n*=5). Following antimicrobial application and a water rinse (50±5 ml), carcasses were drip-dried for one minute and rinsed in 100 ml BPW or nBPW. After rinsing, rinse fluids were collected and subjected to USDA-prescribed *Salmonella* detection procedures.

Results: Only 53.3% of the PAA-BPW-treated carcasses were presumptively positive for *Salmonella*, while all other treatments (PAA-nBPW, CPC-BPW, CPC-nBPW, CPC_{80}-BPW, CPC_{80}-nBPW) returned 100% of samples *Salmonella*-positive. The effectiveness of BPW was lower (P<0.001) than nBPW in capacity to neutralize antimicrobials on broiler carcasses. The rinse fluid by antimicrobial intervention combination BPW-PAA had lower (P<0.001) *Salmonella* detection than all other combinations of rinse fluids and antimicrobials.

Significance: Use of nBPW is anticipated to increase pathogen detection from poultry carcasses treated with antimicrobial interventions, though food safety impacts of nBPW use in routine testing remain unknown.

T11-04 Prevalence of *Salmonella* in Deep Tissue Lymph Nodes of Pork

TINEKE JONES, Cara Service and Scott Hrycauk
Agriculture and Agri-Food Canada, Lacombe, AB, Canada

Introduction: Little attention, to date, is being paid to the prevalence of *Salmonella* in deep tissue lymph nodes (DTLN) in tissues used for ground pork. DTLN could be an important source of *Salmonella*, as carcass decontamination strategies have no effect on *Salmonella* that are deeply embedded and protected. Full removal of pork lymph nodes in specific pork cuts and trimmings is being demanded in a number of international markets.

Purpose: This study was conducted to determine the prevalence and concentration of *Salmonella* in DTLN from the belly, ham, and shoulder and from ground pork.

Methods: A total of 240 DTLN, obtained from the ham (80), belly (80) and shoulder (80) of pork carcasses, and 80 ground pork samples were collected over a 7 month period from a commercial pork processing plant. DTLN were surface sterilized and minced. *Salmonella* were detected and enumerated by standard methods. A 12 ml portion of each homogenized DTLN was stored at 4°C for enumeration by hydrophobic grid membrane filtration in the event the sample was positive by enrichment.

Results: Overall, *Salmonella* was detected in 0.8% (2 of 240) of DTLN by enrichment; one positive sample was detected in a DTLN from the belly and one DTLN from the shoulder. The numbers of *Salmonella* were below the limit of detection by hydrophobic grid membrane filtration. *Salmonella* was not detected in ground pork samples.

Significance: In this study, the prevalence of *Salmonella* in DTLN in pork tissues for human consumption was very low; indicating that it could be a minor source of contamination in the production of ground pork. These findings provide important information for the pork industry to use when assessing the risks and benefits of removing DTLN from pork cuts and trimmings.

T11-05 Evaluation of Transfer Rates of *Salmonella* from Single-use Gloves and Sleeves to Dehydrated Pork Jerky

JIAN WU, Monica Ponder, Thomas Saunders, Kendall Fogler and Kim Waterman
Virginia Tech, Blacksburg, VA

Introduction: Meat jerky is a popular dried snack food that is typically considered shelf-stable and ready-to-eat. A lethality treatment is necessary to control biological hazards. However, many jerky processes incorporate postlethality handling that represents opportunities of contamination through contact with worker hands/gloves and forearms/sleeves.

Purpose: This study identified transfer rates of *Salmonella enterica* from gloves to dried jerky after handling with three types of single-use gloves (nitrile, PVC, and PE) and one type of single-use PE-coated sleeves that covers worker's forearms.

Methods: Six *Salmonella enterica* serovars were mixed and diluted to seven to eight log CFU/ml and two to three log CFU/ml for quantitative and qualitative transfer rate analyses, respectively. For quantitative analysis, high dose inoculum was applied evenly to the palm of the glove (or a defined area of sleeve) and every gloved hand (or sleeve) was used to touch three jerky strips, successively. In total, 18 strips were inoculated per material. For qualitative analysis, low dose inoculum was applied evenly to the palm of the glove and the gloved hand was used to touch the jerky strips (*n*=40) using two contact scenarios (fingers-only or fingers-and-palm contact), simulating activities associated with hand sorting and packaging. *Salmonella* were enumerated by plating onto XLT4 following serial dilution or after 24 h enrichment.

Results: *Salmonella* transfer to jerky was significantly greater (P<0.05) from PE gloves (5.52±0.24 log CFU/sample) and PE-coated sleeves (6.16±0.49 log CFU/sample) compared to nitrile (4.47±0.47 log CFU/sample) and PVC gloves (4.66±0.58 log CFU/sample). In qualitative analysis, fingers-only contact resulted in *Salmonella* transfer to 10 of 40 jerky strips from PE gloves and 1 of 40 strips from nitrile gloves. However, when the palm was involved in the contact, all 80 jerky strips acquired *Salmonella,* regardless of material types.

Significance: Selection of materials associated with reduced transfer may be an important strategy for reducing bacterial cross-contamination in jerky production facilities.

T11-06 Effect of Persistent and Transient Generic *Escherichia coli* and *Salmonella* spp. Recovered from a Beef Packing Plant on Biofilm Formation of *Escherichia coli* O157:H7

Jeyachchandran Visvalingam[1] and **XIANQIN YANG**[2]

[1]*Agriculture Agri-Food Canada, Lacombe, AB, Canada,* [2]*Agriculture and Agri-Food Canada, Lacombe, AB, Canada*

Introduction: Persistence of *Escherichia coli* and related pathogens like *E. coli* O157 on beef packing plant equipment may result from biofilm formation by these organisms. Biofilms are, generally, resistant to regular cleaning and sanitizing and may act as a continuous source of contamination for otherwise unadulterated beef cuts and trimmings.

Purpose: This study examined the influence of persistent and transient generic *E. coli* genotypes and *Salmonella* spp., recovered from a beef packing plant, on biofilm formation of *E. coli* O157:H7.

Methods: Biofilm formation of five genotypes of persistent and transient generic *E. coli* and a strain of *Salmonella* spp. was tested alone or in dual-cultures with *E. coli* O157:H7 at 15°C for up to six days. Biofilms were quantified using Crystal Violet (CV) staining on day 2, 4, and 6. The numbers of *E. coli* O157:H7 in mono- or dual-culture biofilms with persistent and transient *E. coli* genotypes 390 and 533, respectively, and with *Salmonella* spp. were determined by plating on selective agar.

Results: Strain by day interaction was found ($P<0.0001$) when biofilm formation of *E. coli* genotypes and *Salmonella* spp. were cultured alone or with *E. coli* O157:H7. In monoculture biofilm, the numbers of *E. coli* O157:H7 ranged between 5.87 and 6.69 log CFU at ≤ six days. *Escherichia coli* genotype 136 significantly ($P<0.05$) reduced the numbers of *E. coli* O157 in dual-culture biofilms by ≥1.5 log CFU at ≥ two days, while genotype 533 caused ≥ one log CFU reduction at ≥ four days. *Salmonella* spp. did not affect ($P>0.05$) the numbers of *E. coli* O157:H7 in dual-culture biofilm.

Significance: The finding that generic *E. coli* genotypes significantly reduced biofilm formation by *E. coli* O157:H7 suggested that generic *E. coli* may potentially be used as an agent to control *E. coli* O157:H7 in beef processing environment.

T11-07 Changing United States Population Demographics: What Does this Mean for Listeriosis Incidence and Exposure?

AURELIE POHL[1], Regis Pouillot[2] and Jane Van Doren[1]

[1]*U.S. Food and Drug Administration–CFSAN, College Park, MD,* [2]*U.S. Food and Drug Administration, CFSAN, College Park, MD*

Introduction: *Listeria monocytogenes* is an important cause of foodborne illness, hospitalization, fetal loss, and death in the United States. Listeriosis incidence rate varies among population subgroups with pregnant women, older persons, and the Hispanic population having increased relative risks for listeriosis.

Purpose: As the demographic makeup of the United States population changes over time, with respect to ethnicity, pregnancy status, and age distribution, how is the rate of listeriosis expected to change?

Methods: Using estimated rates of listeriosis per subpopulation based on FoodNet data from 2004 to 2009, we evaluated the expected number of cases and incidence rates of listeriosis in the United States population and the pregnant women subpopulation as the demographic composition of the population changes.

Results: If the incidence rate per subpopulation is held constant, the overall United States population listeriosis incidence rate would increase from 0.25 per 100,000 (95% CI: 0.19 to 0.34) in 2010, to 0.28 (95% CI: 0.22 to 0.38) in 2020 and 0.32 (95% CI: 0.25 to 0.43) in 2030 because of the changes in population structure. The pregnancy-associated incidence rate is expected to increase from 4.0 per 100,000 pregnant women (95% CI: 2.5 to 6.5) in 2010, to 4.1 (95% CI: 2.6 to 6.7) in 2020, and 4.4 (95% CI: 2.7 to 7.2) in 2030. We further estimate that a reduction of 12% in the exposure of the population to *L. monocytogenes* would be needed to maintain a constant incidence rate from 2010 to 2020; assuming infectivity (strain virulence distribution and individual susceptibility) is unchanged. To reduce the overall United States population incidence rate by one third (Healthy People 2020 goal) would require a reduction in exposure (or infectivity) to *L. monocytogenes* of 48% over the same time period.

Significance: This information will be useful in setting public health targets, developing risk management options, and in interpreting trends in the public health burden of foodborne listeriosis in the United States.

T11-08 Prevalence and Antibiotic Resistance of *Escherichia coli* and *Enterococcus* spp. in Urban Agriculture

LIYANAGE NIRASHA PERERA, Abdullah Ibn Mafiz and Yifan Zhang

Wayne State University, Detroit, MI

❖ ***Developing Scientist Competitor***

Introduction: Globally, urban farming is gaining popularity as a sustainable agricultural system for providing heathy and inexpensive food. However, there are limited data available on the microbial safety related to this sector of agriculture.

Purpose: This study examined the prevalence and antibiotic susceptibilities of *Escherichia coli* and *Enterococcus* spp. in soil and vegetables associated with urban agriculture.

Methods: Fifteen soil and 48 vegetable samples were collected from three urban gardens in Detroit. *Escherichia coli* and *Enterococcus* spp. were isolated and identified by PCR. Unique isolates were identified as *Enterococcus* spp. by PCR and 16S rRNA sequencing. The disc diffusion test was used to examine the antimicrobial susceptibility profile of all unique isolates of *E. coli* and *Enterococcus* spp. Pulsed-field gel electrophoresis (PFGE) was performed to distinguish the bacteria at the molecular level.

Results: Out of 15 soil samples, 9 (60%) carried *E. coli* and 14 (93%) contained *Enterococcus* spp. Of 48 vegetable samples, 23 (48%) were positive for *E. coli* and 38 (79%) for *Enterococcus* spp. *Enterococcus mundtii* (47%) was the most abundant *Enterococcus* spp. in soil, followed by *Enterococcus durans* (20%) and *Enterococcus casseliflavus* (13%). In contrast, the vast majority of *Enterococcus* spp. from vegetables was identified as *Enterococcus faecalis* (82%). *Escherichia coli* showed resistance to ampicillin, only, in 33% of soil and 61% of vegetable samples. Streptomycin resistance was observed in 64% of *Enterococcus* spp. from soil, followed by 7% to ciprofloxacin. Streptomycin resistance was the most prominent in *Enterococcus* spp. in vegetables (79%). Further, resistance to ampicillin (32%), ciprofloxacin (8%), erythromycin (8%), and tetracycline (8%) was observed in *Enterococcus* spp. of vegetable origin. PFGE results demonstrated a diverse population of *E. coli*, but no unique patterns identified in isolates from soil or vegetables.

Significance: Common foodborne bacteria are prevalent in the urban agricultural system and may serve as a vital source of food contamination and antibiotic resistance.

T12-01 Microbiological Safety of Chicken Sold in Flow Pack Wrappers

THOMAS OSCAR

U.S. Department of Agriculture-ARS, Princess Anne, MD

Introduction: The flow pack wrapper is a popular packaging choice for whole chickens. However, it may provide a favorable environment for growth and spread of *Salmonella* within the package leading to an outbreak of salmonellosis.

Purpose: This study investigated a model that was developed to predict the risk of salmonellosis from chicken parts prepared from whole chickens, sold in flow pack wrappers, and subjected to proper storage (6 h at 4°C) or improper storage (72 h at 15°C) before preparation.

Methods: The model consisted of four unit operations (pathogen events): i) preparation (contamination); ii) cooking (death); iii) serving (cross-contamination); and iv) consumption (dose-response). Data for prevalence, number, and *Salmonella* serotype on chicken parts were obtained by whole sample enrichment, real-time polymerase chain reaction.

Results: Improper storage increased ($P<0.05$) prevalence of *Salmonella* on raw chicken parts from 10.6% (17 of 160) to 41.2% (66 of 160) and on cooked chicken parts from 10% (4 of 40) to 52.2% (24 of 46). Mean numbers of *Salmonella* increased ($P<0.05$) from 0.003 to 3.47 log on raw chicken parts and from 0.006 to 3.15 log on cooked chicken parts after improper storage. Predominant serotypes isolated ($n=111$) were Typhimurium (34.2%), Typhimurium var 5- (20.7%), Kentucky (12.6%), Enteritidis (11.7%) and Heidelberg (8.1%). When chicken was properly stored before preparation, the model predicted that risk of salmonellosis was low and sporadic. However, when 0.1 to 1% of chicken was improperly stored before preparation, the model predicted that risk of a salmonellosis outbreak increased ($P<0.05$) linearly.

Significance: These results demonstrated that the flow pack wrapper provided a favorable environment for growth and spread of *Salmonella* within the package and that even when only a small percentage of packages were subjected to improper storage before preparation, risk of a salmonellosis outbreak increased significantly.

T12-02 Using a Quantitative Risk Assessment on Norovirus Transmission in Food Establishments to Improve and Prioritize the Implementation of Control Measures

GIRVIN LIGGANS[1], Wendy Fanaselle[1], Steven Duret[1], Efstathia Papafragkou[2], Regis Pouillot[3], Laurie Williams[1] and Jane Van Doren[1]

[1]U.S. Food and Drug Administration, College Park, MD, [2]U.S. Food and Drug Administration, Laurel, MD, [3]U.S. Food and Drug Administration, CFSAN, College Park, MD

Introduction: Most norovirus foodborne illness in the United States is from contamination of food by infected food employees in retail food establishments. The FDA Food Code has addressed the control of norovirus transmission from ill food employees to RTE food since 2005, but the incidence of foodborne norovirus has not declined since these provisions were first published. A recently published quantitative risk assessment model on the transmission of norovirus in retail food establishments provides the FDA with a new tool for the evaluation of preventive measures, based on employee compliance.

Purpose: This study investigated the use of this model as a new tool in prioritizing and implementing Food Code provisions to control the transmission of foodborne norovirus.

Methods: The model is used as a tool in evaluating foodborne norovirus control strategies. Results from the model are evaluated with food employee compliance to identify and focus on critical controls necessary to have an impact on foodborne norovirus in the United States.

Results: The model results support the current recommendations of the FDA Food Code and highlight the importance of food employee compliance with many of these strategies. Results show these prevention strategies could reduce, but not prevent norovirus transmission to food when a symptomatic employee is present in the food establishment. Full compliance with all Food Code interventions reduces the mean level of infected customers to 30.3 (90% variability interval=0,116) and the mean number of sick customers to 0.3 (90% variability level=0.0, 1.7). In contrast, no compliance with exclusion measures increases infected customers to 167.4 (90% variability interval=29.0, 357.7) and ill customers to 5.2 (90% variability interval=0.1, 17.2) for every 2,000 food servings.

Significance: Study results showed that using this model to evaluate Food Code guidelines can provide new insights for implementation and maximum impact on reducing norovirus foodborne illness.

T12-03 Modeling for Predicting the Growth of *Salmonella* in Chicken Fillets under Different Temperatures

HAIYING PANG[1], Wen Wang[2], Xingning Xiao[1], Jianming Zhang[3], Ming Liao[3] and Yanbin Li[4]

[1]Zhejiang University, College of Biosystems Engineering and Food Science, Hangzhou, China, [2]Institute of Quality and Standard of Agricultural Products, Zhejiang Academy of Agricultural Sciences, Hangzhou, China, [3]South China Agricultural University, Guangzhou, China, [4]University of Arkansas, Department of Biological and Agricultural Engineering, Fayetteville, AR

Introduction: *Salmonella* has been considered as one of the major pathogens associated with poultry around the world. Various empirical models have been developed for predicting the growth of *Salmonella* in chickens. However, the comparison of these models under different temperatures is needed for quantitative microbial risk assessment.

Purpose: The objective of this study was to compare the performance of empirical models for predicting the growth kinetics of *Salmonella* in chicken fillets under different temperatures.

Methods: Chicken fillets (size: 2cm by 1cm by 1cm; weight: 2±0.3g) were inoculated with three-strains of cocktail *Salmonell*a (ATCC 14028, 50335, 51957) at the initial contamination level of four to five log CFU/g, and then stored at temperatures of 13, 16, 25, 33, and 37°C. Growth data were collected for fitting into four primary models; namely modified Gompertz model, Huang model, Buchanan linear model, and Baranyi model. Three secondary models (Arrhenius-type model, Ratkowsky square root model, and Huang square root model) were selected for describing the maximum growth rates derived from primary models. Statistical indices of R2 and RMSE were used for model evaluation. Independent trials were conducted, and Bias factors (Bf) and Accuracy factors (Af) were calculated for model validation.

Results: The modified Gompertz model described growth data the best, followed by Huang model. The average maximum growth rates of *Salmonella* in chicken fillets were 0.076, 0.116, 0.582, 0.687, 0.785/h at 13, 16, 25, 33 and 37°C, respectively. R^2 for Arrhenius-type model describing the maximum growth rate obtained from the modified Gompertz model was 0.99, which was the best among selected secondary models (RMSE=0.047, B_f=1.01, A_f=1.04).

Significance: The selected model was able to predict the growth of *Salmonella* in chicken fillets under different temperatures in processing and storage conditions, which may be used in quantitative microbial risk assessment.

T12-04 Growth of *Salmonella* Enteritidis in Liquid Egg Whites During Refrigerated Storage and Temperature Abuse: A One-step Dynamic Analysis

LIHAN HUANG

U.S. Department of Agriculture-ARS, Wyndmoor, PA

Introduction: *Salmonella* Enteritidis is a foodborne pathogen that affects the safety of eggs and egg products. Egg-associated salmonellosis is a serious public health problem in the United States and many parts of the world. Understanding the growth and survival of *Salmonella* Enteritidis in egg products may help prevent and reduce foodborne salmonellosis.

Purpose: This study was conducted to investigate the growth and survival of *Salmonella* Enteritidis in liquid egg whites.

Methods: Pasteurized liquid egg white samples were inoculated with a five-strain cocktail of *Salmonella* Enteritidis and incubated under dynamically changing temperature conditions between 2 and 37°C to observe the growth and survival of the bacterium. Two differential equations were used in combination with the Huang square-root secondary model to describe the growth and survival of *Salmonella* Enteritidis. A one-step dynamic analysis method was used to determine the kinetic parameters using least squares optimization by minimizing the sum of squared residuals.

Results: No lag phase was observed in the growth curves. The growth and survival of *Salmonella* Enteritidis was accurately analyzed using the one-step dynamic analysis method. The estimated minimum growth temperature (T_{min}) of the bacterium was 7.76°C, matching well with the general characteristics of *Salmonella* Enteritidis. At temperatures below T_{min}, the bacterial would die of at 0.136 log CFU/g per day per °C. For model development, the root mean square error (RMSE) was only 0.43 log CFU/g. About 70% of the residual errors were within 0.5 log CFU/g of observations. For validation, about 69% of the residual errors were within 0.5 log CFU/g of observations.

Significance: This study demonstrated that the one-step dynamic analysis is an effective method for investigating the growth and survival kinetics of foodborne pathogens. The results obtained from this study can be used to predict the growth of *Salmonella* Enteritidis in liquid egg whites and to conduct risk assessment of this pathogen.

T12-05 Evaluating a Demonstration-based Training Model for Educating Environmental Health Specialists on Validation and Verification of HACCP Plans

VERONICA BRYANT[1], Natalie Seymour[2], Katrina Levine[2] and Benjamin Chapman[2]

[1]NC Dept of Health & Human Services, Raleigh, NC, [2]North Carolina State University, Raleigh, NC

Introduction: Environmental health specialists (EHS) are tasked with inspecting regulated food businesses that in some jurisdictions are required to develop, submit, and follow HACCP plans for some specialized processes. To properly prepare the EHS community for these regulatory requirements, a two-day, hands-on training program for HACCP plan review and inspection was developed.

Purpose: This project was carried out to determine if the course is successful at increasing knowledge and skills and improving attitudes of regulators. Online surveys were used to evaluate the effectiveness of a demonstration and participation based course on validation and verification of retail HACCP plans.

Methods: Surveys were sent out to 262 participants from courses offered in North Carolina between 2014 and 2016. Respondents were asked to self-identify attitude, skills, abilities, and comfort level with the material before and after completion of the course. Specific questions were asked regarding course material to measure retention of course knowledge.

Results: Of 44 respondents, 58% indicated their knowledge increased greatly after the course and 46% indicated their comfort level increased greatly. After completing this course, 47% reported identifying a previously unidentified specialized process occurring in an establishment. Eighty-one percent ranked themselves as knowledgeable or very knowledgeable and able to implement the knowledge. There was a 32% increase in participants who strongly agree they could recognize acidification and fermentation and a 39% increase for reduced oxygen packaging.

Significance: Results suggested that this course model is an effective method for presenting new material to local and state regulators. The course provided participants with knowledge that was implemented by regulators to improve food safety of specialized processes. This course can be used nationwide to increase knowledge and skills regarding validation and verification of HACCP plans.

T12-06 Analysis of Certified Food Protection Manager Examination Results after a New Training Approach

NATALIE SEYMOUR and Benjamin Chapman

North Carolina State University, Raleigh, NC

Introduction: The 2013 United States FDA Food Code prescribes that all retail and food service businesses have at least one employee, who is a certified food protection manager, present at all times during operation. Since behavior and attitude change must occur to increase food safety, a curriculum model was created to make the material more accessible to food employees. This curriculum was based in case studies and grounded in behavior change and adult learning theories.

Purpose: This study evaluated examination (exam) data from 24 months of exams administered to participants of this program. Researchers sought to identify trends in mastery of exam content in order to guide curriculum revisions. Increased mastery of concepts could influence confidence in material and better understanding could help promote behavior change.

Methods: Aggregate data from 760 exams was collected from the National Registry of Food Safety Professionals and analyzed for trends by month, proctor, and category of questions. Percentage of correct answers in every category was used to determine level of proficiency: mastered, competent, and needing review.

Results: The exam pass rate was 81%, with an average scaled score of 82 out of 100. Diagnostic data revealed 30% of examinees needed review in time and temperature concepts and 28% in personal hygiene. Preventing contamination and facility maintenance had the highest level of mastery, 50% and 46%, respectively.

Significance: The results of this study showed the areas needing more attention in the food safety training program and served as the first step in assessing behavior change. The categories needing the most improvement are among the top contributing factors for foodborne illness, so increased mastery of these concepts could improve compliance to food safety standards.

T12-07 Evaluation of Grocery Store Food Safety Audits for Patterns in Handwashing and Temperature Compliance

Natalie Seymour[1], **THOMAS FORD**[2], Eric Laber[1], Joyce Cahoon[1] and Benjamin Chapman[1]
[1]North Carolina State University, Raleigh, NC, [2]Ecolab Inc., Greensboro, NC

Introduction: Food safety auditing at food grocery stores is often conducted to assess food safety practices. Audits are tailored to grocery store chains' needs and priorities, such as temperature control or adherence to employee hygiene. Questions, duration of audit, and departments visited may differ between chains. Data from eight companies was included, spanning 2009 to 2015.

Purpose: This study was carried out to provide insight into handwashing and temperature violations throughout the deli, meat, bakery, seafood, produce, and general departments and to test for trends in noncompliance. Differences in compliance based on geographic location, time of day, or year may reveal gaps and factors in food safety that were previously unknown. This knowledge could influence changes to training and infrastructure to improve compliance to food safety standards.

Methods: The data set comprised of 72,278 unique store audits and 9.5 million data points. Questions from 17 unique audit forms, targeting the same category of violation, were analyzed as a unit. Data was geocoded and fit into pairwise associations between violations and geographic, temporal, and departmental differences. A logistic regression model was fit to determine any differences between the 336 auditors.

Results: Handwashing violations were highest in deli departments (0.0745) and throughout the East North Central Division of the United States. Temporal differences had small effects in handwashing compliance, but proportions of temperature violations were highest in the morning (0.204), on Tuesdays (0.171), and during the months of August to October (0.180, 0.179, 0.173). Temperature violations were highest in produce (0.371) and meat (0.129) departments.

Significance: Data on the temporal, geographic, and departmental trends allowed greater insight into whether violations were related and predicted by internal and external factors. Results will be used to identify priority areas for targeted intervention strategies and continued research to identify other factors in noncompliance.

T12-08 Food Safety Knowledge and Practices and Consumers' Willingness to Pay for Fresh-cut Produce

HEYAO YU, Jack Neal and Sujata A. Sirsat
University of Houston, Houston, TX

Introduction: The Centers for Disease Control and Prevention (CDC) identified 713 fresh-cut produce-associated outbreaks between1990 and 2005. Due to the frequent outbreaks related to fresh-cut produce, consumers' food safety perception may have changed. However, no previous study has been conducted to investigate consumers' risk concerns toward fresh-cut produce and their willingness to pay (WTP) for improved safety attributes.

Purpose: This study aimed at investigating (i) consumers' knowledge and practices for handling fresh-cut produce, (ii) risk concerns toward fresh-cut produce, and (iii) factors influencing their WTP for improved safety attribute.

Methods: A survey instrument was designed to collect consumers' input on their fresh-cut produce handling knowledge and practices, risk concerns of fresh-cut produce, and their WTP for improved food safety in fresh-cut produce. A total of 1,043 respondents participated in this nationwide survey. ANOVA, logistic regression, and linear regression were used for data analysis in the study.

Results: Respondents lacked knowledge of storage order (CR= 9.5%), surface cleaning (CR= 27%), and disposing of fresh-cut produce (CR= 34%). The risk concern of pesticide was significantly higher than other factors ($F(6,6348) = 56.90$, $P<0.001$). In addition, the majority (64.2%) of respondents agreed to pay a premium to enhance food safety practices. The results of logistic and linear regression indicated that risk perceptions (B=0.217, $P<0.05$), high income level (B= 0.591, $P< 0.05$), age (B= 0.477, $P< 0.01$), and purchasing frequency (B= 0.357, $P< 0.05$) increased the probability of consumers' WTP for improved food safety attributes.

Significance: The results showed that more effective education materials related to fresh-cut produce should be developed to increase consumers' awareness and knowledge about fresh-cut produce food safety. Also, the results encourage retail and restaurant operators to consider increasing revenue by investing in food safety.

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Poster Abstracts

P1-01 Detecting Thermal Inactivation of Human Norovirus on Spinach Using Propidium or Ethidium Monoazide Combined with Real-time Quantitative Reverse Transcription-polymerase Chain Reaction

MYEONG-IN JEONG, Shin Young Park, Seh Eun Kim, Rui Mei-ling, Heedae Park and Sang-Do Ha
Advanced Food Safety Research Group, Brain Korea 21 Plus, Chung-Ang University, Ansung, South Korea

Introduction: Human norovirus (HuNoV), as a serious viral agent, is the main cause of acute gastroenteritis diseases worldwide. An obstacle in HuNoV research concerns the inability of the virus to be cultivated in all environments. Propidium monoazide (PMA) and ethidium monoazide (EMA) can be applied, as a pretreatment, prior to real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) to distinguish between live and dead cells.

Purpose: In Korea, spinach is mostly cooked by blanching, followed by seasoning with soy sauce and other condiments to manufacture muchim. Therefore, the present study aimed to evaluate the inhibitory efficacy of heat against HuNoV in suspension (6 to 85°C, for one minute) and on spinach (6 to 95°C, for two minutes) by applying PMA- or EMA-pretreatment combined with qRT-PCR (PMA/qRT-PCR or EMA/qRT-PCR).

Methods: HuNoV stock samples, in 500 µl aliquots (approximately seven log copy number/µl), were heated for one minute in a water bath set at 65, 75, or 85°C. Spinach samples, which were inoculated with 1,500 µl of HuNoV suspension, were heated for two minutes in a water bath at 65, 75, 85, and 95°C. The inactivation effects of thermal treatment against human norovirus (HuNoV) in suspension and on spinach was evaluated by using qRT-PCR, EMA/qRT-PCR, and PMA/qRT-PCR.

Results: Total titers of non-dye treated, EMA-treated, or PMA-treated HuNoV in suspension were significantly (P<0.05) reduced to 0.22 to 0.77, 0.42 to 2.42, and 0.54 to 2.96 log copy number/µl, respectively, after thermal exposure at 65 to 85°C for one minute. HuNoV titers on spinach were, also, significantly (P<0.05) reduced to 0.27 to 1.01, 0.34 to 2.39, and 0.82 to 2.59 log copy number/µl in qRT-PCR, EMA/qRT-PCR, and PMA/qRT-PCR, respectively, after treatment at 65 to 85°C for two minutes.

Significance: HuNoV, in suspension or on spinach, treated with PMA or EMA exhibited a greater decrease compared with nondye treatments at all heating temperatures.

P1-02 Withdrawn

P1-03 The Effects of Electron Beam Irradiation on the Inactivation of Murine Norovirus-1 in Abalone Meat and Viscera

SEH EUN KIM, Rui Mei-ling, Shin Young Park, Jiyeon Jo and Sang-Do Ha
Advanced Food Safety Research Group, Brain Korea 21 Plus, Chung-Ang University, Ansung, South Korea

Introduction: Shellfish is considered a major source of norovirus (NoV) outbreaks. Because abalone, a type of shellfish, is popular as a raw and stamina food in East Asia, including Korea, there has been an increase in the consumption of this food. Therefore, studies to control of NoV in abalone treated with nonthermal processes are urgently required. However, there is a lack of study on the antiviral effect by electron beam irradiation (e-beam) as one of the nonthermal processes. The FDA allows e-beam irradiation up to 5.5 kilogray (kGy) to decrease foodborn microorganisms on the shellfish.

Purpose: This study was conducted to evaluate the effects of e-beam irradiation (1 to 0 kGy) on inactivation of murine norovirus-1 (MNV-1), as a NoV surrogate, in cell culture lysate and abalone meat and viscera.

Methods: The cell culture lysate (7.63 log plaque forming unit (PFU)/ml of MNV-1) and abalone meat and viscera (7.63 log PFU/ml of MNV-1) were irradiated by e-beam (1, 3, 5, 7, and 10 kGy). The titer of MNV-1 was measured by plaque assay. The physical characteristics (Hunter colors and textures) were also observed as quality parameters. D-values (90% reduction) of e-beam were calculated using the first-order model.

Results: The titer of MNV-1 significantly decreased to 0.40 to 2.08, 0.31 to 1.45 and 0.41 to 1.56 log PFU/ml in suspension and abalone meat and viscera, respectively, as the dose of e-beam irradiation increased. D-values correspond to 4.67 kGy in suspension (R^2=0.99), 5.64 kGy in meat (R^2=0.99) and 4.75 kGy in viscera (R^2=0.99), respectively. Hunters colors and textures were not significantly different in abalone following e-beam irradiation.

Significance: These data suggests that a 5.64 and 4.75 kGy of e-beam irradiation dose was sufficient to reduce 90% of MNV-1 in abalone meat and viscera, respectively, without any deleterious changes in food qualities. Thus, e-beam could be regarded as an effective nonthermal treatment to reduce MNV-1 in abalone.

P1-04 Efficacy of Hypochlorite Bleach Treatment on Different Human Norovirus Genotypes

Flor Maes[1], Genesis Chavez Reyes[2], Giselle Almeida[2] and **KRISTEN GIBSON**[2]
[1]University of Ghent, Ghent, Belgium, [2]University of Arkansas, Fayetteville, AR

Introduction: Human norovirus (hNoV) is the primary cause of foodborne illnesses in the United States. This group of viruses are highly infectious and very diverse making it hard to control. The most effective disinfectant is hypochlorite bleach while other disinfectants such as ethanol have minimal impact depending on the hNoV genotype.

Purpose: The objectives of this study were to evaluate the efficacy of hypochlorite bleach against different GI, GII, and GIV hNoV genotypes in suspension and to determine differences in susceptibility between genotypes.

Methods: The hNoV genotypes (n=11) were suspended separately in a bleach solution (50, 100, or 150 ppm) for up to five minutes followed by neutralization with fetal bovine serum. The neutralized suspension was treated with RNAse. The RNA of the intact viruses was extracted and analyzed by RT-qPCR. To determine log reduction, cycle threshold (Ct) values from untreated and treated hNoV were compared and expressed as change in Ct value.

Results: Analyses have been completed for 100 and 150ppm. Following five minutes exposure at 100 and 150ppm, GII.4 Sydney and New Orleans, GII.7, and GI.1 showed no change compared to untreated virus while a less than one-log reduction was observed for GI.5, GI.6, and GIV. Conversely, GII.3, GII.6, GII.13, and GII.16 displayed complete inactivation (three- to four-logs) at both 100 and 150 ppm after one minute exposure.

Significance: An hNoV genotype dependent efficacy of bleach inactivation is important for practical application of inactivation research. Studies using only one or a limited number of genotypes might not convey the full spectrum of hNoV susceptibility or resistance.

P1-05 Thermal Stability of Viruses in Coculture with *Enterobacter cloacae*

KRISTEN GIBSON and Giselle Almeida
University of Arkansas, Fayetteville, AR

Introduction: Human noroviruses (hNoV) are the primary cause of foodborne disease in the United States. Thermal stability has been studied though there is limited data on the impact of bacteria-virus interactions on the stability of enteric viruses such as hNoV. It is presumed that viruses associated with environmental materials are physically more stable than nonassociated viruses.

Purpose: This study aims to investigate the thermal stability of viruses when associated with *Enterobacter cloacae*.

Methods: Tulane virus (TuV), murine norovirus (MNV), and Aichivirus (AiV) were selected for thermal stability evaluation along with *E. cloacae*. For each experiment, 400 µl 10^5 PFU/ml of virus was added to 400 µl 10^9 CFU *E. cloacae/ml* and exposed to 37, 56 and 63, and 72°C for up to 24 h, 30 min, and 10 min, respectively. To detect virus at 37°C not associated with *E. cloacae*, samples were filtered prior to analysis by plaque assay. At ≥56°C, *E. cloacae*was inactivated by ≤ one minute; thus, both filtered and whole samples were analyzed at specific time intervals.

Results: Analysis of AiV has been completed. At 37°C, AiV remained stable at five logs for 24 h and was fully associated with *E. cloacae* by 6 h postinoculation. At 56°C, AiV with and without bacteria was completely inactivated at 10 min with *D*-values of 41 and 43 s, respectively. Five-log inactivation of AiV with and without *E. cloacae* was achieved by 30 s at 63 and 72°C. TuV and MNV experiments are ongoing.

Significance: The use of viral surrogates is critical in applied food safety research related to thermal inactivation. Often researchers study pathogens in monoculture, and this is not representative of the "real-world". These data are the first step in understanding the role that microbe-microbe interactions may play in survivability.

P1-06 Rapid Association of Enteric Viruses with Whole Cell Bacteria in Suspension

Giselle Almeida and **KRISTEN GIBSON**
University of Arkansas, Fayetteville, AR

Introduction: The association of enteric viruses such as human noroviruses (hNoV) with bacterial cells has been suggested as an important relationship for survival and perhaps infectivity. Viruses recognize various host cell receptors including histo-blood group antigens (HBGAs), and bacteria have been reported to express HBGA-like substances.

Purpose: This study aims to investigate the binding capacity of Tulane virus (TuV), murine norovirus (MNV), and Aichivirus (AiV) with whole cell bacteria at room temperature (RT).

Methods: Here, 400 µl of 10^7 PFU/ml AiV, 10^6 PFU/ml TuV, and 10^7 PFU/ml MNV were incubated individually with 10^9 CFU bacteria for up to two hours at RT. Bacteria used include *Pantoea agglomerans*, *Pantoea ananatis*, *Enterobacter cloacae*, *Bacillus cereus*, *Pseudomonas* spp., and *Exiguobacterium sibiricum*. Unbound viruses were recovered by filtration and detected by plaque assay using the corresponding host cells. Interaction of viruses with bacterial cells was visualized by transmission electron microscopy (TEM).

Results: Data suggest that both AiV and TuV readily associated with all bacterial species within 10 min at RT with complete viral association after two hours. There was a significant difference (P<0.0001) in the % mean of unbound TuV (29±15%) and AiV (3.7±2.0%) after 10 min, but no differences thereafter. There were also differences in binding activity to specific bacteria within virus type. For instance, % TuV not bound to *E. sibiricum*—a gram-positive lettuce isolate—was significantly higher than other bacteria at most time points. The same trend was observed with AiV as well as lower % binding of both viruses with *B. cereus*. TEM confirmed virus association with bacterial cells. MNV analysis is ongoing.

Significance: These data demonstrate a time dependent interaction of select cultivable viruses with both gram-negative and -positive bacterial cells. Understanding these interactions will allow further studies in the role these relationships play in virus survivability.

P1-07 Impact of Moisture Content and Temperature during Rice Storage on Levels of Mold and Aerobic Bacteria over Time

KRISTEN GIBSON, Giselle Almeida, Wenjun Deng, Bhagwati Prakash, Shweta Kumari and Terry Siebenmorgen
University of Arkansas, Fayetteville, AR

Introduction: Rice (*Oryza sativa*) in the Mid-South United States is typically stored at 11 to 13% (wet basis) moisture content (MC). Lacking standard practices, some rice is over-dried resulting in lower rough rice (RR) mass and higher drying costs. Conversely, under-drying could favor microbial growth (mold, aerobic bacteria) and cause quality reductions.

Purpose: This study aims to determine the impact of storage MC and temperature on concentrations of mold and aerobic plate count (APC) in RR stored over a 12-month period.

Methods: Long-grain rice (cultivar Roy J) was harvested at three locations from Northern to Southern Arkansas. Composite samples were divided, dried to 12, 13, 14, or 15% MC, and stored at 25 or 35°C for up to 12 months. To determine total mold and APC at harvest, after drying, and at specified storage durations, both surface elution (SE; hand massage) and total elution (TE; stomacher) methods were applied to duplicate 25 g RR samples. Eluents were collected, diluted, and plated on 3M Petrifilm for Mold/Yeast and APC and incubated per the manufacturer's instructions.

Results: Analyses have been completed through two months of storage. Total elution yielded ~0.47 and ~0.94 log CFU/ml greater mold and APC counts, compared to SE. Regardless of the MC level, storage at 35°C yielded significantly (P≤0.015) lower mean log CFU/ml mold (3.28±0.66) and APC (5.73±0.59) compared to 25°C with 4.43±0.35 and 6.75±0.44 log CFU/ml mold and APC, respectively. Overall, the higher MC levels of RR stored at 35°C yielded lower microbial concentrations for all harvest locations compared to 12%. Analysis of ≥4 months storage is ongoing.

Significance: Determination of optimal storage conditions for rice is imperative to decrease potential food safety issues (toxigenic molds) and quality issues (color, milleability, milling yields). Additionally, less energy intensive practices related to drying could increase sustainability within the rice industry.

P1-08 Effect of Plant Proteases on Infectivity of Tulane Virus, Murine Norovirus, and Hepatitis A Virus

ADRIENNE SHEARER and Kalmia Kniel
University of Delaware, Newark, DE

Introduction: Plant proteases, papain, bromelain, and ficin have application in the meat, brewing, and dairy industries and have shown bacteriostatic, antifungal, antihelminthic, and antitoxin effects. Bromelain exerts antiviral activity by cleaving influenza virus surface antigens.

Purpose: The effect of bromelain, papain, and ficin on cell-culture infectivity of Tulane virus (TV), murine norovirus (MNV), and hepatitis A virus (HAV) were evaluated.

Methods: TV, MNV, and HAV were propagated in LLCMK2, RAW 267.4, and FRhK4 cells, respectively, in the absence of serum, filtered (0.2 µm pore) from cell debris, and treated in duplicate trials (10^4 to 10^5 PFU/ml initial concentration) by individual and combined proteases (2500 ppm with 2 mM cysteine-HCl) in HBSS (pH 7) at 45°C for 60 min, 50°C for 10 min, 55°C for 10 min and in citrate buffer (pH 5.5) at 50°C for 10 min. MNV was treated with bromelain at 50°C for various times up to 15 min. Virus infectivity was enumerated by plaque assay or $TCID_{50}$. Controls included untreated virus, virus heated without proteases, and uninoculated cell culture media and protease solutions.

Results: MNV infectivity was reduced by one and 2.5 logs by papain and bromelain, respectively, at 45°C for 60 min and 3 logs by bromelain at 50°C for 10 min compared to heated and untreated controls ($P<0.05$). The reduction in MNV at 50°C could be attained within 6 min; no greater reduction was realized with 15 min treatment. HAV infectivity was reduced (one-log) with the combined proteases at 55°C for 10 min ($P>0.05$). No reduction in TV infectivity was observed. Detection sensitivity was 10 PFU/ml.

Significance: Plant proteases would make desirable antiviral agents for enhanced safety of raw, ready-to-eat produce. Susceptibility of the viruses to the plant proteases suggests variable enzyme access to receptor-binding moieties or sufficient peptide bonds to affect capsid integrity and warrants investigation of human norovirus.

P1-09 Persistence of Murine Norovirus in Vegetable Wash and Brackish Tidal Surface Waters

ADAM VANORE, Adrienne Shearer, Samantha Gartley and Kalmia Kniel

University of Delaware, Newark, DE

❖ ***Developing Scientist Competitor***

Introduction: Drought and flooding have increased interest in use of non-traditional agricultural waters for irrigation which may become contaminated with or transport enteric viruses.

Purpose: To assess viability of vegetable wash and brackish tidal surface waters in survival and detection of enteric viruses. Here, methods for the evaluation of murine norovirus (MNV) persistence were assessed for future evaluation of water samples.

Methods: Samples (200-L) of vegetable wash and brackish tidal surface waters were collected from two sites in the Mid-Atlantic and subsamples, in duplicates, were inoculated with MNV (5.0-log PFU/ml) as a surrogate for human norovirus, and stored at 12°C. RNA was extracted from each sample on the day of inoculation and days 1, 3, 6, and 9 and stored at -20°C. RNA was evaluated by qPCR and compared to MNV standard curves. Aerobic plate counts were evaluated on uninoculated wash and surface waters using TSA incubated at 37°C overnight.

Results: Over nine days, MNV persisted in each sample, each day, at concentrations consistent with the original inoculum concentration (4.0 log PFU/ml) as compared to standard curves created with each qPCR run. Ct values were as follows: 25.22 for days 0 and 1, 21.72 for days 3 and 6, and 23.25 for day 9. Ct values for all samples were within acceptable range of their corresponding standard curve, where R^2 values were 0.99. Aerobic plate counts revealed bacterial concentrations ranging from 5.96×10^2 to 5.63×10^2CFU/ml for the two vegetable wash water samples and 18.3 to 41.7 CFU/ml for the two brackish tidal surface water samples.

Significance: Neither bacterial populations nor quality/chemical characteristics interfered with qPCR. MNV persisted in surface and vegetable wash waters collected in Fall, 2016, allowed survival of MNV, warranting further studies on human enteric virus detection and survival.

P1-10 Optimization of Virus Recovery from Non-porous Surfaces with Application in Environmental Persistence Studies

NICOLE TURNAGE and Kristen Gibson

University of Arkansas, Fayetteville, AR

Introduction: Human noroviruses (HuNoVs) are the leading cause of foodborne illness in the United States. Contamination of fomite surfaces is a major route of transmission. To better understand virus persistence on fomite surfaces under various conditions, application of an optimized virus recovery method is critical.

Purpose: This study aims to optimize a surface sampling method for virus recovery from nonporous surfaces (NPS) with application in environmental persistence studies.

Methods: Tulane virus (TuV) and Aichivirus (AiV) were selected for NPS sampling optimization. One hundred microliters of virus (10^4 to 10^6 PFU/ml) was inoculated onto duplicate NPS (plastic, stainless steel, acrylic) and dried under ambient conditions for one hour. Viruses were recovered using one of three implements (cell scraper, repeated pipetting, or macrofoam swab) combined with one of two eluents (1× phosphate buffered saline (PBS) or 1× PBS + 0.1%Tween 80 (PBST, 1:1 v/v)). For persistence studies, HuNoVs (GI.1; GII.17) and Aiv were evaluated. Virus-inoculated NPS were placed into an environmental chamber at varying temperatures (6°C, 15°C, 22°C) and relative humidity (RH; 60%, 90%) and recovered at specified time points over two-weeks. Virus recovery efficiency and persistence were obtained through plaque assay or RT-qPCR.

Results: The repeated pipetting with PBST method was selected though no significant differences were observed compared to other methods. Recovery efficiencies ranged from 22.1±14.9% to 82.6±38.6% depending on virus and NPS type. Data indicate HuNoV GI.1 is more stable than GII.17 on NPS at 22°C/60%RH with GI.1 detection over 14 days and no signal detected for GII.17 at 3 days. For AiV, a three-log reduction was observed over 14 days on NPS at 22°C/60%RH. Virus persistence at remaining temperature-RH combinations is ongoing.

Significance: Evaluation of surface sampling techniques is typically limited to swabs for application in environmental sampling during HuNoV outbreaks. An optimized virus recovery method for laboratory-based persistence studies has been described here allowing for uniformity across studies.

P1-11 Survival of Norovirus Surrogates, Feline Calicivirus, and Murine Norovirus on Carpets

DAVID BUCKLEY, Angela Fraser, Guohui Huang and Xiuping Jiang

Clemson University, Clemson, SC

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Introduction: Epidemiological evidence suggests soft surfaces, such as carpet, may be a route of transmission for human norovirus (HuNoV), a leading cause of foodborne disease. To date, no studies have determined survival characteristics of HuNoV on surfaces.

Purpose: We aimed to determine survival of HuNoV surrogates on hard and soft surfaces under environmentally relevant conditions.

Methods: Shaved wool carpet fibers, nylon carpet fibers (0.1 g), and glass coverslips were inoculated with HuNoV surrogates, feline calicivirus (FCV) strain F9 and murine norovirus (MNV) strain 1 at a titer of ca. six log pfu/sample then held at 30% or 70% relative humidity (RH) at 25°C. Surrogates were recovered from carpets using 0.01M PBS + 0.02% Tween 80 in our mini-spin column method and from glass using a previously published method at selected sampling dates. Surrogates were quantified using standard plaque assay and RT-qPCR.

Results: FCV survived for up to 15, 3, and 3 days at 30% RH on wool, nylon, and glass, respectively, and up to 7, 1, and 0 days at 70% RH on wool, nylon, and glass, respectively. MNV survived for up to 15, 7, and 7 days at 30% RH on wool, nylon and glass, respectively, as compared to 7, 3, and 3 days at 70% RH on wool, nylon, and glass, respectively. The highest titer reduction ranged from 3 to <5.3 log pfu/sample when surrogates were enumerated by plaque assay. Minimal reduction (<1.1 log over 60 days) in surrogate genomic copies as determined by RT-qPCR was observed for both environments, excluding glass at 70% RH.

Significance: This is the first study to determine survival of HuNoV surrogates on carpet under varying environmental conditions. Results indicate HuNoV surrogates can survive on carpet suggesting carpets could be an exposure source to HuNoV.

P1-12 Development and Evaluation of Nucleic Acid Aptamers to a Novel Target Protein for Treatment and Detection of Human Norovirus

MATTHEW MOORE, Jeremy Faircloth and Lee-Ann Jaykus
North Carolina State University, Raleigh, NC

Introduction: One of the challenges for rapid control and detection of human noroviruses is their high degree of diversity, which makes generation of broadly-reactive ligands difficult, as these ligands target the viral capsid proteins. Noroviruses contain a more widely conserved protein, VPg, covalently linked to the 5′ end of the ssRNA genome. VPg is necessary for replication of the virus as it recruits host cell translation machinery at the beginning of the viral replication cycle, but has not been a main focus for generation of therapeutics or ligands for detection. Nucleic acid aptamers are emerging ligands that have shown promise as therapeutics and ligands for numerous viruses.

Purpose: The purpose of this study was to generate nucleic acid aptamers targeting the VPgs of human Norwalk virus (NV) and Tulane virus (TV), a cultivable surrogate.

Methods: The VPg regions of TV and NV were cloned into *Escherichia coli*, overexpressed, and used as targets for selection with aptamer pools containing a 40 nucleotide variable region. After aptamers were selected, their Gibbs energy values (DGs), secondary structures, and shared sequence motifs were evaluated. The aptamers effectiveness at reducing viral replication for TV were evaluated by plaque assay.

Results: After eight rounds of selection, 5 and 10 unique sequences out of 17 and 21 sequence pools (n) for TV and NV were identified, respectively. Numerous unique secondary structures with overlapping motifs were identified, with DGs ranging from -7.79 to -18.07 and -9.75 to -18.13, suggesting sequences form stable structures. Both TV (76%) and NV (38%) pools had a predominant sequence, suggesting good selection; and one similar sequence was shared between pools. Plaque assays showed TV VPg aptamers were dose-dependent, with $\log_{10}$reductions ranging from 0.12 to 1.05, which is comparable to other published therapeutic virus aptamers.

Significance: This work demonstrates the potential of a hitherto, under-investigated, protein target for human norovirus treatment and detection.

P1-13 Detection of Human Norovirus in Fermented Food Using a Conductive Polymer Coated Magnetic Separation Combined with Quantitative Reverse Transcription-PCR

JEEHYOUNG HA, Sung Hyun Kim, Su-Ji Kim, Hee Min Lee, Hae-Won Lee, Ji-Hyun Lee and Jae Yong Lee
World Institute of Kimchi, Gwangju, South Korea

Introduction: Outbreaks of viral diseases are frequently associated with the consumption of nonthermally fermented foods. Among the viruses in these outbreaks, human norovirus (NoV) has been reported as the most common foodborne virus. This study aimed to evaluate the recovery of structurally intact NoV during kimchi fermentation.

Purpose: The aim of this study was to attempt to recover structurally intact human NoV, in order to determine the survival of human NoV.

Methods: In this study, a method for the detection of NoV GII.4 in fermented kimchi using a conductive polymer coated magnetic separation combined with Quantitative Reverse Transcription-PCR was developed. Intact viral particles were captured using anionic magnetic beads, which were prepared by the grafting of poly (poly(MVE-MA)) in a dimethyl sulfoxide (DMSO)/phosphate buffer solution. A zeta potential and particle size analyzer was used to measure the zeta potential value of 200 nm magnetic beads coated with anionic polymer.

Results: Our conductive, polymer coated, magnetic beads exhibited zeta potential values between -28.80 and -29.48 mV, demonstrating a negative charge due to coating of the beads with the anionic polymer. Based on conductive polymer coated magnetic beads combined with qRT-PCR results, recovery rates of NoV GII.4 from PBS solution (control) and kimchi samples inoculated with NoV GII.4 were 68.71±3.28% and 41.95±7.38%, respectively. These results verified that GII.4 can be detected in fermented foods by using the conductive polymer coated magnetic beads combined with qRT-PCR method.

Significance: A conductive polymer coated magnetic separation technique removes PCR inhibitors and reduces time-consuming concentration steps for the concentration of virus particles in kimchi samples. Recovery sensitivity achieved using our method was similar to that of other concentration methods such as polyethylene glycol (PEG)-precipitation and ultrafiltration. These results demonstrate that the conductive polymer coated magnetic beads combined with qRT-PCR assay can be used to efficiently and rapidly detect viruses in various food samples.

P1-14 Norovirus Contamination on Environmental Surfaces during Norovirus Outbreaks on Cruise Ships, 2015 to 2016

GEUN WOO PARK[1], Amy Freeland[1], Nikail Collins[2], Aimee Treffiletti[1] and Jan Vinjé[1]
[1]Centers for Disease Control and Prevention, Atlanta, GA, [2]Atlanta Research and Education Foundation (AREF), Atlanta, GA

Introduction: Norovirus is the leading cause of acute gastrointestinal enteritis (AGE) outbreaks on cruise ships. Environmental contamination of highly touched surfaces, likely, plays an important role in the transmission of the virus, but has not been well studied on cruise ships.

Purpose: We aimed to determine the norovirus bioburden on highly touched surfaces, on six cruise ships, during norovirus outbreaks and to assess the disinfection efficiency of several disinfection procedures.

Methods: Swab samples were collected from highly touched surfaces (*n*=95) in 19 AGE case cabins, as well as from surfaces (*n*=110) in public areas on the disembarkation day of six cruise ship voyages. After cleaning and room disinfection, the same surfaces in 16 case cabins were again sampled to determine norovirus reduction. Norovirus RNA was extracted using lysis buffer and concentrated using spin columns. RNA preparation was followed by realtime RT-PCR assays for detection and quantification of norovirus titers.

Results: Norovirus was detected in 43 (45.3%) of the 95 swab samples from case cabins and from 30 (27.3%) of the 110 samples from public areas. Viral titers ranged from 1.2 to 7.4 log RNA copies for the case cabin samples and from 1.6 to 2.7 log RNA copies for the public areas. Toilet seats in cabins showed the highest norovirus contamination rates 67% (12 of 19) with a titer range of 4.3 to 6.8 log RNA copies. Average norovirus reduction titers, on cabin surfaces after disinfection, was 0.4 log. Different disinfection measured on the ships resulted in a modest reduction of norovirus RNA levels (≤1.6 log). There were no illnesses reported in those cabins on the subsequent voyage.

Significance: This is the first study to characterize the level of environmental contamination on cruise ships during norovirus outbreaks. Overall, our data help to explore a framework for evidence-based norovirus outbreak management on cruise ships.

P1-15 Evaluation of the Efficacy of Copper (100% Cu) and Brass (70% Cu) for Inactivation of a Human Norovirus Surrogate by Porcine Gastric Mucin Binding and Infectivity Assays

JORDAN RECKER and Xinhui Li
University of Wisconsin-La Crosse, La Crosse, WI

❖ ***Developing Scientist Competitor***

Introduction: Human noroviruses (HuNoVs) are the leading cause for foodborne illnesses. Copper and copper alloys have been shown to inactivate many pathogens, including HuNoVs. Due to the difficulty in culturing HuNoVs, studies of inactivation of HuNoVs usually rely on surrogates and assays based on qRT-PCR. However, the accuracy of using assays, based on RT-qPCR, to quantify infectious virus particles for copper and copper alloy treatments is still unclear.

Purpose: This study evaluated the efficacy of copper (100% Cu) and brass (70% Cu) for inactivation of Tulane virus (TV), a suitable surrogate for HuNoV, by both plaque assay and porcine gastric mucin-conjugated magnetic beads (PGM-MB) binding assay followed by RT-qPCR (referred to as PGM-MB/PCR assay).

Methods: TV was inoculated onto and treated on copper and brass coupons for 0, 10, 20, 30, and 40 minutes (three replicates). The virus was eluted at the time points. Eluted viruses were assayed for infectivity by plaque assay or treated by RNase A, bound by PGM-MB and quantified by RT-qPCR (PGM-MB/PCR assay).

Results: As assessed by plaque assay, a 20-minute treatment by copper achieved a 4.2±0.3 log reduction (P<0.05) of TV, close to the maximum detectable reduction by the assay (4.3±0.2 log reduction). However, no more than 1.0-log reduction was observed from all the copper treatments when PGM-MB/PCR assay was used. Brass treatment for 40 min, only, reduced TV by 1.5±0.3 log (P<0.05) by plaque assay, while for the PGM-MB/PCR assay with the same treatment, no reduction (0.0±0.0 log, P>0.05) was observed.

Significance: These results suggest that copper can rapidly inactivate TV. The PGM/PCR assay would likely underestimate the efficacy of copper and copper alloys for inactivation of HuNoVs.

P1-16 Comparison of Filtration Methods to Recover *Cyclospora cayetanensis* Oocysts from Agricultural Water Samples

ALEXANDRE DA SILVA[1], Mauricio Durigan[1], Helen Murphy[1], Amy Kahler[2], Mia Mattioli[2], Jannifer Murphy[2] and Vincent Hill[2]
[1]U.S. Food and Drug Administration–CFSAN, Office of Applied Research and Safety Assessment, Laurel, MD, [2]Centers for Disease Control and Prevention, Division of Foodborne, Waterborne and Environmental Diseases, Atlanta, GA

Introduction: Filtration methods, such as USEPA Method 1623, used for the recovery of parasite oocysts and cysts, play an essential role in confirming or ruling out irrigation water as a potential vehicle for contamination of produce. However, there is limited information regarding the comparative performance of Method 1623, relative to other large volume sampling methods for recovery of the parasite, *Cyclospora cayetanensis*, from agricultural water samples.

Purpose: This study compared the CDC's dead-end ultrafiltration method (DEUF) with the use of Envirocheck microfilters per Method 1623 for recovery of *C. cayetanensis* from irrigation pond water with various turbidities. Both methods have been widely used for recovery of Cryptosporidium oocysts and Giardia cysts, but have not yet been validated for *C. cayetanensis*.

Methods: Two sets of 50-L agricultural water samples that represented low and high turbidity (0.71 and 21.6 NTU average, respectively) were each seeded with approximately 162 oocysts/liter and processed using both filtration methods. A qPCR assay, validated by the U.S. FDA for the detection of *C. cayetanensis* in produce, was used to quantify *C. cayetanensis* in the concentrates. The experiments were repeated five times for each filtration method.

Results: For both DEUF and Method 1623, *C. cayetanensis* was detected in five of five high and low turbidity water samples.

Significance: This comparative study demonstrated that the DEUF method performance was not significantly different than Method 1623 Envirocheck filtration in the recovery of *C. cayetanensis* oocysts from agricultural water with various turbidity levels. However, the DEUF is less expensive and less prone to clogging, allowing for processing of larger and lower quality water samples.

P1-17 Molecular Characterization of *Cyclospora cayetanensis* in Produce and Clinical Samples Using Whole Mitochondrial Genome Sequencing

HEDIYE CINAR[1], Gopal Gopinath[2], SeonJu Choi[2], Jeongu Lee[2], Sonia Almeria[1], Mauricio Durigan[1], Helen Murphy[1] and Alexandre da Silva[1]
[1]U.S. Food and Drug Administration–CFSAN, Office of Applied Research and Safety Assessment, Laurel, MD, [2]U.S. Food and Drug Administration, Laurel, MD

Introduction: *Cyclospora cayetanensis* is a coccidian parasite causing large outbreaks in different countries. In the United States, outbreaks affected 1,481 people from 2013 to 2015. Outbreak investigations are limited by the absence of molecular epidemiological tools for tracebacks. Because of difficulties in the recovery of the oocysts from produce, the unculturable nature of the organism, and limitations in efficient DNA extraction due to resistant oocyst wall structure, it is extremely challenging to generate sufficient DNA templates for robust amplification of large DNA fragments, used in traditional sequencing analysis.

Purpose: In different apicomplexan parasites, multicopy organellar DNA, such as mitochondrion genomes, have been used for detection and molecular epidemiology analysis. We developed a workflow, to obtain complete mitochondrial genome sequences from contaminated foods and clinical samples, for differentiation of *C. cayetanensis* isolates.

Methods: The 6,274 bp long *C. cayetanensis* mitochondrial genome, in four overlapping amplicons from genomic DNA, extracted from cilantro and spiked with oocysts and extracted from clinical stool samples, was amplified by PCR. DNA sequence libraries of the gel-purified amplicons were prepared using the Ovation Ultralow System library kit (NuGEN Technologies) and sequenced using MiSeq. Sequence reads were assembled using CLC Genomics WorkBench, and Geneious programs, to map to a reference *C. cayetanensis* mitochondrial genome.

Results: This approach allowed us to sequence complete mitochondria genomes from produce samples seeded with *C. cayetanensis* oocysts, and from stool samples. SNP profiles of 30 stool samples, from a 2014 *C. cayetanensis* outbreak, exhibited discriminatory power based on geographical metadata. Isolates from different states grouped together in an evolutionary tree, suggesting that genomic analyses of mitochondria sequences may help to link outbreak cases to the source.

Significance: The described method will facilitate the application of genomics tools to epidemiologically link *C. cayetanensis* identified in clinical and food samples during outbreak investigations.

P1-18 Comparison of Detection of *Cyclospora cayetanensis* in a Variety of Food Matrices

SONIA ALMERIA[1], Alexandre da Silva[1], Mauricio Durigan[1], Tyann Blessingnton[2] and Helen Murphy[1]
[1]U.S. Food and Drug Administration–CFSAN, Office of Applied Research and Safety Assessment, Laurel, MD, [2]U.S. Food and Drug Administration, Laurel, MD

Introduction: *Cyclospora cayetanensis* is a major cause of diarrheal illness and outbreaks; however, microbiological sampling and surveillance is limited due to the lack of validated methods for produce. In 2016 a method for detection of *C. cayetanensis* in cilantro and raspberries was validated by the U.S. FDA for regulatory testing. In 2016, a restaurant-associated subcluster of cyclosporiasis illnesses were epidemiologically linked to consumption of cole slaw containing shredded carrots and cabbage. Therefore, it is imperative to evaluate the performance of this validated method in different matrices.

Purpose: This study evaluated the validated method to detect *C. cayetanensis* in commodities, other than cilantro and raspberries, and compared the results.

Methods: A total of 25 grams shredded carrots and cilantro and 50 grams raspberries were seeded with 5, 10, 20, and/or 200 *C. cayetanensis* oocysts from Nepal. Unseeded produce were used as negative controls. The method included washing of the produce, extraction of *C. cayetanensis* DNA, and molecular detection using a TaqMan assay targeting the 18S rRNA gene.

Results: Detection rates in shredded carrots were 26.7% of 15 samples seeded with 10 oocysts and 56.2% of 16 samples seeded with 20 oocysts. All carrot samples seeded with 200 oocysts (n=11) were positive and all unseeded carrot samples (n=11) were negative. Comparatively, *C. cayetanensis* oocysts detection rates for cilantro and raspberries seeded with 5 and 10 oocysts were 31.3% and 80.0%, and 50.0% and 90.0%, respectively, in a multilaboratory validation study.

Significance: The method was slightly less sensitive for detection of *C. cayetanensis* in shredded carrot samples when compared to the results observed in cilantro and raspberries. This study highlights the importance of evaluating the performance of the *C. cayetanensis* detection method in different food matrices as the U.S. FDA advances with the implementation of such tools for regulatory purposes.

P1-19 Evaluation of 405 nm CW Visible Blue Light as a Means of Inactivating Tulane Virus on Blueberries

DAVID KINGSLEY[1], Rafael Perez[2], Glenn Boyd[3], Joseph Sites[4] and Brendan Niemira[5]
[1]U.S. Department of Agriculture, Dover, DE, [2]U.S. Department of Agriculture-ARS-FSIT, Dover, DE, [3]U.S. Department of Agriculture-ARS-ERRC-FSIT, Wyndmoor, PA, [4]U.S. Department of Agriculture-ARS-FSIT, Wyndmoor, PA, [5]U.S. Department of Agriculture-ARS, Wyndmoor, PA

Introduction: Visible blue light (405 nm) is effective against bacteria, but its potential as a nonthermal intervention for viruses on foods, such as berries that are prone to norovirus contamination, has not been evaluated. Tulane virus (TV) is, now, a common human norovirus surrogate that can be propagated in vitro. Riboflavin is a B vitamin and rose bengal is a food coloring agent. Both chemicals are generally recognized as safe (GRAS).

Purpose: The potential of 405 nm light to nonthermally inactivate TV coating the surface of blueberries was evaluated. The potential of compounds, which enhance singlet oxygen production to promote 405 nm inactivation was also evaluated.

Methods: Blueberries were immersed in TV (approximately 10^6/pfu ml), then air dried for one hour. For enhancer experiments, 0.1% w/v rose bengal or riboflavin was added to virus suspensions prior to contamination of blueberries. Berries were, subsequently, treated with 4.2mW/cm^2 405 nm light for 5 to 30 minutes, rotating blueberries with forceps every one to two minutes to ensure exposure of all surfaces to 405 nm light. To mitigate thermal heating due to the intense light, a dry ice-chilled nitorgen-based cooling system was utilized to ensure that temperatures remained below 35°C at all times.

Results: Five, 10, and 30 min treatments resulted in little or no inactivation of Tulane on blueberries (average log reductions of +0.18, +0.02, and -0.06, respectively). However more substantial inactivation was observed, with riboflavin resulting in an average reduction of - 0.51 logs and -1.01 for rose bengal. It was noted that the addition of riboflavin and rose bengal, in the absence of the 405 nm light treatment, produced some inactivation. Average untreated reductions for riboflavin and rose bengal were -0.13 and -0.66, respectively.

Significance: Results indicate that 405 nm light may have some potential to inactivate viruses, if singlet oxygen enhancers are present.

P1-20 Occurrence of Hepatitis E Virus in Regionally Produced Meat Products and the Meat Processing Environment

ARTUR RZEŻUTKA, Iwona Kozyra and Ewelina Bigoraj
National Veterinary Research Institute, Puławy, Poland

Introduction: Identification of Hepatitis E Virus (HEV) strains in food producing animals and in food originated from infected animals makes the issue of public health protection and food safety more important. Although the presence of the virus has been shown in raw meat products, its occurrence in ready-to-eat (RTE) pork meat and variety meat products subjected to low or moderate heat treatment, as well as in meat processing environment has not been investigated.

Purpose: This study was conducted to detect HEV and viral indicators of fecal contamination in RTE pork meat products and meat processing environment. Additionally, an identification of possible routes of food contamination by viruses during its production was investigated.

Methods: In total, 75 samples of RTE pork meat products representing three food categories (white sausage, black pudding, and pie), 42 liver samples as incoming raw material, and 21 swabs taken from food contact surfaces along meat processing were tested for the presence of HEV and porcine adenovirus (pAdV), as the index virus of faecal contamination. In addition, 10 fecal samples from slaughtered pigs were included in the analyses. After extraction and isolation of viral nucleic acids (ISO/TS method), viruses were detected using duplex real-time PCRs with incorporated internal amplification controls.

Results: HEV RNA was not detected in any of the tested food, liver, or swab samples except from one fecal sample; whereas, pAdV was found in white sausage (4 of 25), black pudding (8 of 25), pie (2 of 25), swab sample (1 of 21), and in all animal faeces.

Significance: The presence of pAdV in tested food could indicate the possiblity that HEV survives the heat treatment process. Nevertheless, the risk of foodborne HEV infection due to the consumption of regionally produced RTE pork meat and variety meat products is considered negligible. Environmental surfaces do not play a significant role in food contamination during production and processing.

P1-21 The Use of an Atmospheric Cold Plasma Jet to Inactivate *Cryptosporidium parvum O*ocysts on Cilantro

SHANI CRAIGHEAD[1], Adrienne Shearer[1], Sarah Hertrich[2], Glenn Boyd[3], Joseph Sites[4], Brendan Niemira[2] and Kalmia Kniel[1]
[1]University of Delaware, Newark, DE, [2]U.S. Department of Agriculture-ARS, Wyndmoor, PA, [3]U.S. Department of Agriculture-ARS-ERRC-FSIT, Wyndmoor, PA, [4]U.S. Department of Agriculture-ARS-FSIT, Wyndmoor, PA

❖ ***Developing Scientist Competitor***

Introduction: In 2015, the CDC reported a rise in outbreaks linked to parasites like *Cryptosporidium*. *Cryptosporidium parvum* outbreaks have been associated with contaminated drinking or recreational water; however, there is growing concern that oocysts may become a more common contaminant in foods, especially in produce and herbs, which have been identified as transmission vehicles of protozoan illness.

Purpose: This study examined the effects of a cold plasma jet on viability of *C. parvum* oocysts on fresh cilantro.

Methods: Oocysts (6.25 x 10^5 oocysts/per 500 µl PBS) were applied to glass slides (controls) or fresh cilantro, and treated in duplicate. The 90 min air-dried samples were treated for 0, 30, 90, and 180 s with atmospheric cold plasma jet and placed into 50 ml conical tubes containing 15 ml PBS. Samples were placed on a rotating shaker plate for 30 min and inverted at 15 min, then centrifuged for 20 min at 2,000 x g, treated with 2% bleach, and triple rinsed. Recovered oocysts were subjected to excystation, in duplicate per sample. Oocysts were treated with a 0.75% taurocholic acid/0.25% trypsin solution, incubated at 37°C for 45 mins, and observed. Percent excystation was determined using differential interference contrast microscopy of 100 fields, counting oocyst ghosts, and observing moving sporozoites compared to non-viable oocysts. Data were analyzed using one-way ANOVA.

Results: Data analysis indicated a decrease in percent excystation of oocysts that was statistically significant between the untreated oocysts with >85% excystation and treated (180 s) samples on glass (P=0.0060). Oocysts recovered from cilantro showed significant differences in excystation, between untreated and treated (90 s, 180 s) samples (P<0.01). Treated oocysts showed varying percent excystation at <67%.

Significance: Oocyst excystation is the initial step in infection. Data suggests cold plasma treatment effects *C. parvum* oocyst viability. These results indicate potential inactivation and warrant use of cell culture infectivity assays coupled with variation in oocyst inoculum levels and treatment parameters.

P1-22 Development of PCR Amplification Methods Based on *Cyclospora cayetanensis* Mitochondrial Genomes

MAURICIO DURIGAN[1], Gopal Gopinath[2], ChaeYoon Lee[2], Hediye Cinar[1], Sonia Almeria[1], Helen Murphy[1] and Alexandre da Silva[1]
[1]U.S. Food and Drug Administration–CFSAN, Office of Applied Research and Safety Assessment, Laurel, MD, [2]U.S. Food and Drug Administration, Laurel, MD

Introduction: A method to detect *Cyclospora cayetanensis* in produce was recently validated for regulatory testing by the FDA. This method, which relies on a sample preparation step followed by a TaqMan assay designed using the parasite's 18S rDNA, detects approximately 10 *C. cayetanensis* oocysts in produce. Recent advances in genome sequencing of *C. cayetanensis* include a repository for genome assemblies created in collaboration with NCBI (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA316938), which is being populated with organelle as well as nuclear genome sequences. These novel genetic markers must be evaluated to advance the development of diagnostic detection of *C. cayetanensis*.

Purpose: We designed PCR primers based on *C. cayetanensis* mitochondrial genome sequences and compared them with the validated qPCR method for the detection of *C. cayetanensis* in produce.

Methods: A total of eight PCR primers that produced amplicons ranging from 182 to 538 base pairs were designed. Specificity was tested using DNA extracted from *Eimeria acervulina,* cilantro, and raspberries. Sensitivity was evaluated using DNA extracted from cilantro and raspberries seeded with 10, 20, and 200 oocysts of *C. cayetanensis*. Unseeded cilantro and raspberries were used as negative controls.

Results: All PCR assays from unseeded samples were negative. Two of the eight PCRs amplified *C. cayetanensis* as well as *E. acervulina*. The remaining six PCRs amplified *C. cayetanensis* only; furthermore, one of the PCRs detected as few as 10 oocysts in produce, comparable to the detection limit of the regulatory method.

Significance: This investigation reports the first *C. cayetanensis* molecular detection method designed using mitochondrial genome sequences. This unique tool can be used to confirm the results of the qPCR *C. cayetanensis* method. Studies such as this, support public health and the FDA mission by developing secondary confirmatory methods to support findings requiring regulatory actions.

P1-23 A New RT-Real-time PCR Method for Simultaneous Detection of Hepatitis A Virus, Norovirus (GI, GII), and MS2 Phage in Food and Water Samples

Arnt Ebinger, **OLAF DEGEN**, Cordt Groenewald and Kornelia Berghof-Jager
BIOTECON Diagnostics, Potsdam, Germany

Introduction: Noroviruses (NoV) and hepatitis A virus (HAV) are the leading cause of viral transmitted foodborne disease worldwide. Transmission occurs through consumption of contaminated food and water or through direct contact with an infectious person, leading to severe gastrointestinal or liver diseases. Prominent food-associated outbreaks have been reported lately in Germany (RKI, 2012) and in Europe (EFSA, 2014).

Purpose: The purpose of this study was to validate a new one-step multiplex RT-Real-time PCR-kit for simultaneous detection and differentiation of HAV, NoV (GI, GII) and MS2 phage as a process control. The kit was developed and optimized for the special needs in food and water analytics, regarding inhibiting substances and low-dose contamination in soft fruits, minced meat, seafood and drinking water.

Methods: Virus concentrations from food (berries, mussels, and minced meat) and water samples were carried out according to ISO/TS 15216. RNA was prepared by the foodproof Sample Preparation Kit IV. Virus detection was performed with the foodproof Hepatitis A virus plus Norovirus (GI, GII) Detection Kit.

Results: Norovirus- and hepatitis A virus-positive samples were tested. Specificity was controlled by several norovirus and hepatitis A virus genotypes. The sensitivity of the assay was analyzed by quantified full-length viral RNA and the LOD was set at 10 copies per reaction for all targets. No quenching effects were observed, in case of possible mixed infection. The integrated process control enabled the calculation of recovery rates up to 0.01%.

Significance: The foodproof Hepatitis A virus plus Norovirus (GI, GII) Detection Kit is the first for easy detection in a single assay including a process control. The kit is based on primers, probes, and methods which are mentioned in the ISO/TS 15216.

P1-24 Survival of Hepatitis A Virus on Strawberries under Freeze Drying and Room Temperature Storage

YAN ZHANG[1], Runan Yan[1], Christina K. Carstens[2], David Laird[2] and Y. Carol Shieh[2]
[1]*Illinois Institute of Technology, Chicago, IL,* [2]*U.S. Food and Drug Administration, Bedford Park, IL*

❖ ***Developing Scientist Competitor***

Introduction: Outbreaks caused by viral contamination of berries have been frequently documented. A 2016 hepatitis A virus (HAV) outbreak, consisting of 134 cases in nine states, was attributed to imported frozen strawberries. The minimal process of freeze-drying is desired to preserve fruit sensory quality, but the effectiveness of this process for the inactivation of HAV is unclear.

Purpose: The survival of HAV on strawberry slices was studied during 24 h freeze-dry process (radiant heat ≤40°C) and subsequent storage of dehydrated products at room temperature.

Methods: Fresh strawberries were sliced (7 mm-thickness), inoculated with 5 log PFU HAV/sample, air dried 20 min, frozen at -80°C for 1 h, and freeze-dried 24 h. The strawberry freeze-drying was conducted in FreeZone 12 (Labconco, Kansas City, MO) in multiple trials. HAV survival on strawberry slices was determined by plaque assay using FRhK-4 cells grown in six-well dishes.

Results: Trials averaged 60±14% and 21±9% for total recovered HAV from inoculated berries immediately after air-drying for 20 min and 24 h-cold storage, respectively. The HAV reduction on strawberries after 24 h freeze-drying (with <40°C radiant heat) was ≤0.5 log, compared to corresponding berries 24 h-cold stored as controls. HAV seemed to survive greater than previously studied murine norovirus (MNV, ≤1.8-log reduction) during berry freeze-drying, although similar recoveries of HAV and MNV from fresh and cold stored berries were observed. This freeze drying process did not cause major HAV inactivation on berries. Further investigation on HAV survival on dehydrated berries during room temperature storage will be conducted.

Significance: This research illustrates the persistent nature of HAV on contaminated berries during freeze-drying with radiant heat to 40°C. Thus, preharvest and harvest controls of fruits, intended to precede this type postharvest process, are critical for minimizing viral hazards.

P1-25 Differential Virus Recoveries from Contaminated Abiotic Surfaces

RUNAN YAN[1], Rutuja Khadye[1], Karl Reineke[2], Ashutosh Sharma[1], Timothy Duncan[2] and Y. Carol Shieh[2]
[1]*Illinois Institute of Technology, Chicago, IL,* [2]*U.S. Food and Drug Administration, Bedford Park, IL*

❖ ***Developing Scientist Competitor***

Introduction: Polymers have been commonly used to manufacture containers or tools used for handling foods. These abiotic surfaces could serve as pathogen reservoirs for potential transmission of viruses to foods.

Purpose: The goal is to evaluate virus recoveries from inoculated abiotic plastics such as polyethylene (PE), polypropylene (PP), and polyvinyl chloride (PVC) with smooth or rough surfaces.

Methods: Smooth polymer sheets were made by injection molding, and some were mechanically abraded with sanding paper to produce roughened surfaces. Virus surrogate MS2 suspension was used for inoculation. To arrive at similar drying times, the virus sample was split into two to three aliquots to enhance drying for more hydrophobic surfaces. Inoculated sheets were air-dried (minimum 30 min) and cold stored 24 h before virus elution. The elution started by shaking the sheets twice in an eluent for 10 min at 10°C. Viruses in eluates were filtered through 0.22-µm-pore-size filters before being plaque-assayed.

Results: Among 10 trials comparing the virus recoveries from glass, PVC, and PE surfaces, no significant differences via ANOVA were observed in the recovery immediately after inoculation and one minute drying. The drying time of virus inoculum seemed to impact the recovery: longer drying time resulted in a lower percentage of virus recovered. With controlled drying, the recoveries from smooth high density PE or PP surfaces (after 24 h cold storage) were greater than those from the same materials after roughening with sand paper (up to 50% more recoveries from smooth surfaces, $P<0.05$). To understand any potential recovery change by the surface charge of viral capsid proteins, the virus suspension with alternative pH will be tested in additional experiments.

Significance: The chemical and physical characteristics of abiotic polymer surfaces are illustrated. The data may assist in selecting appropriate food contact surfaces for potentially reduced virus retention and transmission.

P1-26 Isolation and Characterization of *Bacillus cereus* Bacteriophages from Foods and Soil

HYEJIN OH[1], Dong Joo Seo[2], Su Been Jeon[3], Hyunkyung Park[3], Suntak Jeong[3], Hyang Sook Chun[4], Mihwa Oh[5] and Changsun Choi[6]
[1]*Chung-Ang University, Ansung-si, Korea, Rebublic Of,* [2]*Chung-Ang University, Food & Nutrition, Anseong, Kyounggi, South Korea,* [3]*Chung-Ang University, Anseong, South Korea,* [4]*Korea Food Research Institute, Sungnam, Kyonggi, South Korea,* [5]*Rural Development Administration, Jeonju, South Korea,* [6]*Chung-Ang University, Ansung, Kyounggi, South Korea*

Introduction: *Bacillus cereus* is a gram-positive, rod-shaped, sporeforming foodborne pathogen. Since the spore of *B. cereus* is resistant to heat, acid, ultraviolet, gamma irradiation, water stress, it is widely isolated from soil, sediment, dust, plants, and food production environments. Bacteriophage has the significant advantage in inhibiting specific pathogens without alteration of organoleptic properties. Several *B. cereus* bacteriophage strains have been isolated from mud, food waste, and fermented foods. However, potential risk of bacteriophage to carry virulence genes was not examined.

Purpose: This study aimed to isolate bacteriophages against *B. cereus* from various origins and to characterize host range, virulence profiles, and lytic activity of isolated bacteriophages.

Methods: Twenty-seven bacteriophages against *B. cereus* were isolated from various Korean traditional fermented foods and soils. Plaque size, transmission electron microscopy, virulence profile, and in vitro lytic activity of bacteriophage isolates were examined. Transmission electron microscopy confirmed *B. cereus* bacteriophages belonging to the family *Siphoviridae*.

Results: Among *B. cereus* bacteriophages with broad host range, 18 isolates (66.7%) did not harbor *B. cereus* virulence factors. *Bacillus cereus* bacteriophage isolates from fermented foods did not carry virulence genes. Plaque size of each bacteriophage isolate was not dependent on isolation origin. Bacteriophage strain CAU150036, CAU150038, CAU150058, CAU150064, CAU150065, and CAU150066 effectively inhibited *B. cereus* in vitro within one hour with MOI-dependent manner.

Significance: Bacteriophage strain CAU150036, CAU150038, CAU150058, CAU150064, CAU150065, and CAU150066 are considered potential candidates for controlling the contamination of *B. cereus* in food or other applications.

P1-27 Aqueous Extracts of the Underutilized Garcinia Fruit and Pulp Decrease Tulane Virus Infectivity

MANAS SAHOO and Doris D'Souza
University of Tennessee, Knoxville, TN

Introduction: Natural alternate preventive and control strategies are increasingly being studied to prevent and alleviate human norovirus (HNoV) disease symptoms. Tulane virus (TV) is a cultivable HNoV surrogate used to determine antiviral effects. *Garcinia indica* fruit, indigenous to western-ghat and northern regions of India, is rich in antioxidant, anti-diabetic, anti-cancer, anti-ulcer and antimicrobial properties. The antiviral effects of *Garcinia* extracts against TV for application against HNoV illness need to be determined.

Purpose: The objective of this study was to determine the effect of aqueous extracts of *Garcinia indica* whole dried fruit and semidried pulp against TV infectivity at 37°C over six hours.

Methods: TV (200 µl) at approximately seven log PFU/ml was mixed with equal volumes of *Garcinia* fruit and pulp aqueous extracts (50 and 100 mg/ml) or phosphate-buffered saline (PBS, pH 7.2, as control) for 2, 3, and 6 hours at 37°C. After each time interval, treatments were stopped in cell culture media containing 10% fetal bovine serum (FBS) and serially diluted in media containing 2% FBS. Infectivity was determined using standard plaque assays on confluent LLC-MK2 cells in six-well plates in duplicate. Each treatment was replicated thrice and recovered titers were compared to PBS controls.

Results: *Garcinia* pulp extract (100 mg/ml) resulted in TV reductions of 1.7, 2.4, and ~3 log PFU/ml after 2, 3, and 6 h, respectively, while 50 mg/ml showed only ~1.2, 2.1, and 2.2 log PFU/ml reduction after 2, 3, and 6 h, respectively. The dried fruit extract at 100 mg/ml showed 1.1, 1.9, and ~2 log PFU/ml reduction, while 50 mg/ml showed 0.74, 1.7 and 1.9 log PFU/ml reduction after 2, 3, and 6 h, respectively.

Significance: The results indicate that *Garcinia* fruit and pulp aqueous extracts show antiviral effects against TV that are concentration and time-dependent. This study shows the need for further exploration of the potential health benefits of the underutilized *Garcinia* fruit.

P1-28 Recovery Efficiency of Coccidian Parasites from Cilantro Depending on Sample Size and Elution Solution Volume

YNES R. ORTEGA and Maria Torres
University of Georgia, Griffin, GA

Introduction: Outbreaks of cyclosporiasis have been associated with consumption of contaminated cilantro imported from a *Cyclospora*-endemic location in Mexico. Factors influencing the detection of *Cyclospora* and *Cryptosporidium* from cilantro spiked with low number of oocysts have not been fully examined.

Purpose: The objective of this study was to determine the effect of sample size and volume of elution solutions on the recovery of oocysts from experimentally spiked cilantro.

Methods: Cilantro bunches (100 and 250 g) were inoculated with 100 *Cyclospora* and 100 *Cryptosporidium* oocysts. Cilantro bunches were processed at zero and seven days post-inoculation using 100 ml, 250 ml, and 1 liter of 0.1% Alconox or elution buffer (PBS pH 7.0, Tween 80, antifoam). Oocysts were concentrated by centrifugation or by hollow fiber filtration. Triplicate samples representing all combinations of test parameters in two independent trials were analyzed for the presence of oocysts by nPCR targeting the18S rRNA gene.

Results: Oocyst recovery from 100 and 250 g cilantro (spiked with 100 oocysts) previously rinsed with 60 ppm peroxiacetic acid was higher. When samples were prerinsed with chlorine, higher number of positive samples were obtained using Alconox. Higher recoveries were obtained after seven days postinoculation compared to recoveries on the same day of inoculation. Washes using 200 ml detected more positive samples (69%) compared to 500 ml washes (33%). *Cyclospora* oocyst recovery (50%) was higher than *Cryptosporidium* oocysts when using Alconox (18%).

Significance: Testing of foods containing coccidian parasites require multiple wash steps and concentration processes making the methodology tedious and time consuming. Consideration should be given to the sample size, wash solution, and concentration methodologies.

P1-29 Hepatitis A Virus in Scallops Implicated in a 2016 Outbreak

JACQUELINA WOODS[1], Rachel Rodriguez[1], Toni Morales[2], Gilberto Vaughan[3], Yulin Lin[3] and Guo-Liang Xia[3]
[1]U.S. Food and Drug Administration, Dauphin Island, AL, [2]U.S. Food and Drug Administration, Rockville, MD, [3]Centers for Disease Control and Prevention, Atlanta, GA

Introduction: In the United States, hepatitis A (HAV) outbreaks associated with the consumption of molluscan shellfish have not occurred in over 10 years. In addition, there have not been any reports in the United States of HAV infection where scallops were the implicated vehicle. However, in June of 2016, a cluster of HAV illnesses associated with the consumption of imported uncooked bay scallops occurred.

Purpose: The objectives of this analysis were to detect, enumerate, and characterize HAV in bay scallops associated with an outbreak.

Methods: Individually quick frozen (IQF) bay scallops were analyzed for the presence of HAV using a high pH eluent along with ultracentrifugation for concentration and murine norovirus was utilized as an extraction control. Commercial extraction kits and RT-qPCR assays were used for RNA extraction and detection and HAV levels were enumerated utilizing standard curves. Gel electrophoresis of amplicons from the 5′ untranslated region of the HAV genome was used to distinguish between wild type and laboratory strains of HAV. Conventional RT-PCR or RT-qPCR/qPCR and big-dye terminal sequencing of the VP1-2B region of the HAV genome was used for characterization.

Results: HAV was detected at approximately four genomic copies per scallop consumed. Gel and sequence analysis of the amplicons demonstrated that the genotype from the implicated product was HAV 1A, while the laboratory control was HAV genotype 1B. In addition, genetic analysis revealed 100% homology between the scallop and clinical strains.

Significance: Eating contaminated uncooked molluscan shellfish can pose a health risk which can lead to deadly illnesses caused by enteric viruses such as HAV. Previously, only consumption of adulterated oysters or clams had been implicated in HAV associated shellfish outbreaks in the United States. This was the first reported incidence in the United States of HAV associated illnesses due to consumption of bay scallops.

P1-30 Organic Load Impacts the Virucidal Efficacy of Heat and Chlorine against Human Norovirus and Tulane Virus, a Cultivable Surrogate

NAIM MONTAZERI, Eric Moorman, Matthew Moore, Blanca Escudero-Abarca and Lee-Ann Jaykus
North Carolina State University, Raleigh, NC

Introduction: Contamination of surfaces and foods with norovirus commonly occurs by contact with human feces or vomit. Heat and chlorine are widely used for decontamination, but their efficacy is impacted by organic load.

Purpose: This study evaluated the efficacy of chlorine and heat for inactivation of human norovirus and the cultivable surrogate, Tulane virus (TuV), with and without the presence of organic matter.

Methods: Suspension assays were carried out according to American Society for Testing and Materials protocol E1052-11 for a two minute exposure of viruses to sodium hypochlorite (household bleach) and heat. A 20% suspension of human stool confirmed positive for GII.4 Sydney and ultracentrifuged, TuV tested in purified form or suspened in 20% human feces, served as inocula. Virus inactivation was determined using RNase RT-qPCR on both viruses, and by infectivity (plaque) assay for TuV.

Results: There was a >4-log reduction in purified TuV infectivity at 5.0 ppm free available chlorine (FAC), but 300 ppm FAC was needed for the same reduction when TuV was suspended in 20% feces. However, it took 500 and 300 ppm FAC to achieve >4-log reduction in TuV and human norovirus genome copies, respectively, under the elevated organic load. For heat treatment, the infectious titer of purified TuV was reduced by >4 log after 65°C for 2 min, and at 72.5°C for 2 min in fecal suspension. By RT-qPCR, higher temperatures (>87.5°C) were needed to achieve >4-log reduction in purified TuV genome copy, and with organic matter; 100°C was insufficient to reach the same reduction for either GII.4 Sydney or TuV.

Significance: Organic load was protective of viruses exposed to heat or chlorine. Measurement of residual genome copies, especially when the virus is suspended in organic matter, may represent an extreme measure of virucidal efficacy.

P1-31 Quantifying the Destruction of *Salmonella* spp. during a 24-hour Kombucha Brewing Process

CHRISTINE A. ROCK, Daniel Unruh and Sara Gragg

Kansas State University, Olathe, KS

✦ ***Undergraduate Student Award Competitor***

Introduction: Kombucha is a fermented tea that has been consumed for hundreds of years; however, recently kombucha's popularity has risen as consumers shift to more health-conscious beverage options. The Symbiotic Culture of Bacteria and Yeast (SCOBY) operates as a fermentation mechanism to transform sugar in the sweet tea mixture into a carbonated, tangy, acidic beverage that is the hallmark of kombucha. With the rise of home-brews, it is important to investigate safety of the kombucha-brewing process.

Purpose: This study examined the reduction of *Salmonella* spp. during the traditional kombucha home-brewing process.

Methods: Kombucha was prepared using a locally purchased SCOBY and the traditional method, which includes brewing green tea in boiling water, followed by the addition of sugar and SCOBY. Kombucha and SCOBY-free sweet green tea (control) were inoculated with a *Salmonella* cocktail (*Salmonella* Montevideo, *Salmonella* Typhimurim, *Salmonella* Anatum, and *Salmonella* Newport) at a target concentration of 4 log CFU/ml and stored at 24.4°C. At 0, 1 3, 8, and 24 hours, pH of each sample was monitored and *Salmonella* populations were enumerated on Xylose Lysine Tergitol-4 agar.

Results: There were significant effects of treatment (P<0.0001) and treatment*time (P<0.0001), with kombucha harboring significantly reduced *Salmonella* populations compared to the control (P=0.0142) by three hours. Upon reaching 24 h, kombucha harbored 6.7 log CFU/ml (P<0.0001) less *Salmonella* than did the control. Throughout the 24 hour time period, the pH of kombucha and the control was approximately 3 and 7, respectively.

Significance: The kombucha home-brewing process used in this study was effective at reducing *Salmonella* populations to 0.55 log CFU/ml. These data suggest that *Salmonella* contamination of the raw ingredients used in kombucha may be controlled by traditional kombucha brewing practices; however, because brewing practices and the microbiological quality of each SCOBY can vary, additional research is necessary to determine safety.

P1-32 Influence of Sodium Chloride and Calcium Chloride on the Growth and Death of Pathogenic *Escherichia coli* and Lactic Acid Bacteria in Cucumber Brines

FRED BREIDT[1], Robert Price[1], Breanne Burgess[2], Dorothy Dupree[3] and Elizabeth Andress[4]

[1]U.S. Department of Agriculture–ARS, Raleigh, NC, [2]North Carolina State University, Dept. Molecular and Structural Biochemistry, Raleigh, NC, [3]Dept. Food and Nutrition, University of Georgia, Athens, GA, [4]University of Georgia, Athens, GA

Introduction: Sodium and calcium concentrations have been considered an important factor for the quality of fermented vegetable products, but the role of salt in fermentation safety remains unclear.

Purpose: This investigation was undertaken to determine how salt type and concentration influence the growth and death of cocktails of pathogenic *Escherichia coli* strains (STEC), pathogens of concern for acid foods, or *Lactobacillus* species (LAB, including *Lactobacillus plantarum, Lactobacillus brevis,* and *Lactobacillus paracasei*) found in vegetable fermentations.

Methods: Using a cucumber juice medium (CJ), growth rates were determined with a microtiter plate reader for selected NaCl (0 to 1 M) and $CaCl_2$ (0 to 0.6 M) concentrations. Five-log reduction times were determined by incubating cultures in CJ at pH 3.2 with 50 mM lactic acid and plating on Lauria agar (STEC) or 350 mM lactic acid and plating on MRS agar (LAB). Growth and death rates were calculated with custom Matlab software.

Results: For NaCl, growth rates of STEC strains were not significantly different from the no-salt control. However, growth rates decreased for $CaCl_2$ between 0 and 0.2 M and then increased to the level of the no-salt control, up to 0.6 M. The growth rates for LAB strains increased between two- and three-fold as salt concentration increased for both NaCl and $CaCl_2$. *Escherichia coli* strains had five-log reduction times of 20.4 h, 14.1 h, and 16.6 h for 2% NaCl, 6% NaCl, and 1.1% $CaCl_2$, respectively. For LAB, the five-log reduction times with the same salt concentrations were 33.3 h, 31.2 h, and 70.3 h.

Significance: The data indicate that salt type and concentration have relatively little effect on the growth or death of STEC strains. Increasing NaCl or $CaCl_2$ concentration enhanced growth of LAB species; and $CaCl_2$ may enhance LAB survival and fermentation safety.

P1-33 Microbial Safety and Quality Evaluation of Ultraviolet Treated, Cold-pressed, Colored and Turbid, Fruit and Vegetable Beverages

JESSIE USAGA[1] and Randy Worobo[2]

[1]Cornell University, Geneva, NY, [2]Cornell University, Ithaca, NY

Introduction: The growing demand for health-promoting fruit and vegetable juice blends, with improved nutritional and sensory attributes, has prompted the adoption of nonthermal technologies, including ultraviolet (UV) light. Due to safety concerns, FDA mandates that wholesale juice processors shall ensure a five-log reduction of pathogens. Limited studies have explored alternative conditions to overcome the well known limitations of UV when treating colored liquids with high insoluble solids contents.

Purpose: This study addressed the effectiveness of UV light application by a commercial juice-processing unit at a reduced flow rate, to inactivate *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7, and spoilage microorganisms in turbid and colored beverages.

Methods: Inactivation of five strains/serotypes cocktails of pathogenic *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes*, isolated from fruit and vegetable-derived products linked to outbreaks, was determined in seven turbid and colored cold-pressed beverages (apple-lemon, carrot-cucumber-apple-orange, cucumber-apple-lemon, cucumber-spinach-celery-apple-kale-ginger-lemon-parsley, cucumber-fennel-kale-spinach-lime, celery-ap-

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ple-beet-parsley-ginger, and almond milk). Beverages were inoculated to a targeted initial population of 10^7 CFU/ml and treated with a CiderSure 3500 UV reactor at 150 L/h constant flow rate through multiple consecutive passes. Reduction of molds and yeasts, aerobic mesophilic, and lactic acid bacteria was, also, assessed at the cumulative dose that guaranteed a five-log reduction of the most UV-tolerant pathogen in each product. Beverages were physicochemically characterized. Trials were performed in triplicate.

Results: A five-log reduction of the three pathogens was achieved in all beverages at a maximum cumulative UV dose of 12.0±0.6 mJ/cm^2. The dose required to ensure the targeted reduction significantly varied (P<0.05) depending on the formulation, physicochemical properties (pH, soluble solids, acidity, color, and turbidity), and pathogen inoculated. The reduction of aerobic mesophiles, molds and yeasts, and lactic bacteria varied from 0.5 to 3.6, 0.2 to 2.0, and 0.5 to 3.6 log CFU/ml, respectively.

Significance: The proposed nonthermal treatment represents a suitable processing alternative to ensure the safety and extend the shelf life of colored and turbid cold-pressed beverages.

P1-34 Investigation of the Prevalence and Distribution of *Salmonella* in United States Feed Mills

GABRIELA MAGOSSI[1], Natalia Cernicchiaro[2], Steve Dritz[2], Terry Houser[3], Jason Woodworth[3], Cassandra Jones[4] and Valentina Trinetta[1]

[1]Kansas State University, Food Science Institute, Manhattan, KS, [2]Kansas State University - Vet Med, Manhattan, KS, [3]Kansas State University, ASI, Manhattan, KS, [4]Kansas State University, Manhattan, KS

Introduction: *Salmonella* is an emerging pathogen of public health concern. Each year, *Salmonella* infections cost the food industry approximately $2.3 billion and 33% of reported cases are associated with beef, poultry, or pork. This pathogen has the ability to persist in the environment for long periods of time and to resist different temperatures and water activity levels. *Salmonella* presence in feed mill environment is one of the many potential routes for pathogen transmission into the food production chain. Nevertheless, little is known about *Salmonella* prevalence and association in feed mills environment.

Purpose: The objective of this study was to investigate the prevalence and distribution of *Salmonella* in feed mills in different locations of United States.

Methods: Eleven feed mill locations across eight different states were selected for this study and 12 environmental samples were collected within each feed mill representing samples collected from floor dust, equipment surface, worker shoes and finished product. Visits were conducted during the months of October and November 2016. Samples were analyzed following the FSIS guidelines for isolation and identification of *Salmonella*. Environmental data and associations between factors with pathogen prevalence were analyzed using Stata/SE 12.

Results: Of a total of 238 samples collected, 66.0% tested *Salmonella* positive, and among them 69.4% were positive for O group antigens. All sampled feed mills had at least one *Salmonella* positive location. The percentage of positive samples ranged from 41.7 to 100% across feed mills and from 39.1 to 100% across states. Overall, following production flow, the number of positive samples decreased from the initial processing steps towards the finished product, except for the finished product bin boot, where the feed is stored until shipment. In this site 81.8% of samples tested positive for *Salmonella*.

Significance: The results of this study demonstrate the presence of *Salmonella* in feed mills environment across United States and indicate their potential role as vehicle for pathogen transmission and spread into the food production chain.

P1-35 Influence of Amendment Type on Persistence of *Salmonella* Newport in Soil

JUNE TEICHMANN

University of Delaware, Newark, DE

✦ ***Undergraduate Student Award Competitor***

Introduction: *Salmonella* Newport is a foodborne pathogen isolated from several environmental reservoirs on the DelMarVa Peninsula and has been associated with several produce-related outbreaks. Little is known about interactions between *Salmonella* Newport and soil amendments used as fertilizers.

Purpose: The purpose of this study is to determine *Salmonella* Newport persistence and resuscitation in soils containing poultry litter (PL)-based or chemical fertilizer amendments.

Methods: *Salmonella* Newport (rifampicin-resistant) at 4.4 log CFU/pot was inoculated into pots (7.5cm by 5.5cm) containing soil amended with either: i) PL (raw); ii) heat-treated poultry litter pellets (HTPP) or iii) urea (U) (chemical fertilizer) to provide equivalent levels of nitrogen to soil. Inoculated pots were stored in a growth chamber (20°C, 10 h day length). Pots were irrigated weekly (day 0, 7, 13), and three samples of each treatment were taken immediately before and 24 h after irrigation. Bacterial populations were examined over two weeks and enumerated on xylose lysine deoxycholate agar with rifampicin (XLDR). Soil samples were dried to determine moisture content. Recovered populations of *Salmonella* Newport (log CFU/gdw (gram dry weight)) were analyzed using a one-way ANOVA and student's T-test in JMP.

Results: Mean *Salmonella* Newport populations in PL-amended soils were significantly (P<0.05) higher (3.8±0.58 log CFU/gdw) compared to populations in HTPP-amended (1.9±1.8 log CFU/gdw) or U-amended (1.4±1.8 log CFU/gdw) soils. *Salmonella* Newport populations were present but fell below the limit of detection (<0.62 log CFU/g) in HTPP- and U-amended soils on days 6, 7, and 8. However, populations of *Salmonella* Newport resuscitated to 3.74 log CFU/gdw in HTPP-amended soils on day 13, and to 2.99 log CFU/gdw in U-amended soils on day 14.

Significance: These data suggest that soils containing PL may support higher populations of *Salmonella* Newport than HTPP and U-amended soils, but that *Salmonella* Newport populations are dynamic in both U- and HTPP-amended soils under specific conditions.

P1-36 Remediation of Soil Contaminated by *Salmonella enterica* to Expedite Plant or Replant of Vegetables

MULATUA METAFERIA

University of Sydney, Sydney, Australia

Introduction: Fruits and vegetables that are consumed raw have been implicated in a myriad of foodborne outbreaks and as sources of foodborne pathogens. *Salmonella* was the most commonly reported bacterial pathogen, accounting for nearly half of the outbreaks due to bacteria.

Purpose: To assess the effect of environmental factors and soil types on the persistence and survival of *Salmonella* serovars under controlled conditions. Determine which single or combined cover crop-solarisation treatment will facilitate die-off of *Salmonella enterica* in soil so that there is no recontamination associated with the replanting of leafy greens.

Methods: Extraction and enumeration of *Salmonella* from soil samples was performed following cultural methods. Plating and enumeration was done using spiral plater and automatic colony counter respectively. Enrichment was done for all plates with zero-count using enrichment solution to check the presence of residual *Salmonella* cells.

Results: Because of the large number of treatments, only two way interactions between factors manure, soil, temperature, moisture, serovar and the variable time was considered. Tukey's HSD test was performed for multiple comparison testes. For the controlled study, most of the interaction are significant from $P<0.001$ to $P=0.0226$. There was no significant difference among the cover crop treatment in hastening decline of *Salmonella* however; the application of black plastic significantly hastened the decline of *Salmonella* in the field.

Significance: The quality of the research is very relevant to the fresh produce industry in treating *Salmonella* contaminated soil which decreases the possible preharvest contamination of vegetables and increases growers' and consumers' confidence.

P1-37 Factors That Contribute to *Salmonella* Persistence in Field Soil Samples

CLAIRE MARIK[1], Shani Craighead[1], Samantha Gartley[1], Adam Vanore[1], Thais Ramos[1], Manan Sharma[2], Gordon Johnson[1] and Kalmia Kniel[1]

[1]University of Delaware, Newark, DE, [2]U.S. Department of Agriculture–ARS, Beltsville, MD

Undergraduate Student Award Competitor

Introduction: *Salmonella* contamination of raw agricultural commodities may originate from variable sources; however, the impact of external pressures on bacterial survival like use of plastic mulch, ultraviolet (UV) radiation, and moisture is unclear.

Purpose: Factors that contribute to the presence of *Salmonella* including, soil moisture, use of poultry litter (PL), and protection from UV radiation by use of plastic mulch are evaluated.

Methods: Soil samples were collected from 12 field plots, half of which contained PL over 120 days from May to October. Two composite soil samples of 150 g were collected every seven days from each plot; under plastic (UP) or outside of plastic (NP). Samples (10 g) were analyzed for *Salmonella* using modified FDA BAM method, with incubation in Universal Preenrichment broth at 37°C for 24 hours, followed by tetrathionate (TT) broth or Rappaport-Vassiladis (RV) broth for 24 hours at 42°C, after which samples were plated on XLT-4 agar. Moisture content of each soil sample was determined in an oven at 105°C for 24 hours. Statistical analysis conducted using an unpaired t-test.

Results: Control plot samples on day zero were negative for *Salmonella*. Of the 348 analyzed soil samples, 309 where positive for *Salmonella*. Samples enriched using TT were significantly more likely to be positive for *Salmonella* compared to RV ($P<0.00005$). Soil samples collected from UP had significantly higher ($P<0.0001$) moisture than those from NP. Average moisture content for UP samples was 14.09±3.93% and average moisture content for NP was 9.76±5.52%. Moisture content of UP samples from soil with or without PL showed no obvious significant difference in moisture ($P=0.35$). *Salmonella* from UP samples ranged from 0.04 to 3.32 log CFU/g dry weight over the study.

Significance: While it may be intuitive to link soil moisture with *Salmonella* persistence, *Salmonella* survival in field plots is variable subject to other factors, like climate, wildlife, heat, and UV.

P1-38 The Impact of Heavy Rainfall on *Salmonella* Survival and Transport

DEBBIE LEE[1], Timothy Coolong[2], George Vellidis[2] and Karen Levy[1]

[1]Emory University, Atlanta, GA, [2]University of Georgia, Tifton, GA

Developing Scientist Competitor

Introduction: *Salmonella* has been regularly detected in surface water in southern Georgia but many farms in the region use surface water ponds adjacent to produce fields for irrigation. We recently detected *Salmonella* in irrigation ponds, irrigation systems, and produce on a commercial farm in southern Georgia; however, the sources of contamination were unclear.

Purpose: This study investigated the impact of irrigation on *Salmonella* survival in soil and on crops and the ways in which antecedent soil moisture conditions may modify the effect of an extreme rainfall event on *Salmonella* transport through splash and runoff.

Methods: Eight plots (2' by 10') of cabbage were inoculated with avirulent, ampicillin-resistant gfp-marked *Salmonella*. Half of the plots were subject to routine overhead irrigation while the other half of the plots were kept dry for eight days. Plots were reinoculated and then subjected to a heavy rainfall event (four inches/hour) with a rainfall simulator to determine the difference in *Salmonella* concentrations in soil and on cabbage leaves before and after the extreme rainfall event.

Results: *Salmonella* concentrations in soil and crops decreased over eight days with no significant differences between the wet and dry plots. *Salmonella* concentrations in soil and cabbage in both plot types decreased following the heavy rainfall event but were still detectable in samples from both plot types in low concentrations. *Salmonella* concentrations in soil and splash water were higher in the plots receiving routine overhead irrigation plots, although this difference was not statistically significant.

Significance: Study results indicate a potential concern for produce safety because *Salmonella* can persist on crops and in soil and that *Salmonella* can be transported through rainfall splash. Produce contamination risk may be greater when high levels of soil moisture are maintained. This research will help growers mitigate crop contamination risk by elucidating the timing of highest concern for contamination via splash.

P1-39 Microbial Evaluation of Preprocessed and Postprocessed Tomatoes from Florida Packing Houses during 2013 to 2015

Jaysankar De[1], Aswathy Sreedharan[1], **ALAN GUTIERREZ**[1], You Li[2], Jubair Mohammad[1] and Keith Schneider[1]

[1]University of Florida, Gainesville, FL, [2]Beijing Technology and Business University, Beijing, China

Introduction: Prevention of microbial cross-contamination during postharvest handling is an important step to minimize microbial food safety hazards. Dump or flume tanks, which are widely used in Florida packinghouses to transfer/wash tomatoes, are one critical point for spreading cross-contamination.

Purpose: The main objective of this study, conducted during the 2013 through 2015 harvesting seasons in five regions in Florida, was to evaluate the efficiency of postharvest processing of tomatoes in commercial packinghouses.

Methods: Determination of total aerobic plate count (APC), total coliforms (TC) and generic *E. coli* (EC) from 840 composite samples (five tomatoes/sample), both before and after processing, were carried out to accomplish this objective. One hundred ml of 0.1% (w/v) sterile peptone water (Thermo Fisher Scientific, Waltham, MA) was added to sterile sample bags, each containing five tomatoes. Each tomato was rubbed for 60 s. One hundred µl of rinsate from all serial dilutions was spread plated onto plate count agar (Thermo Fisher Scientific, Waltham, MA) for enumeration of APC, and onto CHROMagar™ ECC (DRG International, Inc., Mountainside, NJ) for determining TC and EC.

Results: The least square mean (LSM) value of APC for preprocessed samples (6.5 log CFU/tomato) was highly significantly lower ($P<0.0001$) in postprocessed tomatoes (5.6 log CFU/tomato). The LSM for TC counts were 4.3 and 3.9 log CFU/tomato in preprocessed and postprocessed samples, respectively. Eighty-nine out of 840 (10.6%) and 821 out of 840 (97.7%) samples had TC and EC counts below the detection limit of 1.3 log CFU/tomato. There was no significant difference in the occurrence of EC in preprocessed and postprocessed samples. The APC and TC counts showed highly significant ($P<0.0001$) seasonal variation.

Significance: Information from this study is suitable for identifying areas in which improvements are necessary to optimize standard postharvest operational procedures to ensure produce safety and subsequently control disease outbreaks.

P1-40 Assessment of Generic *Listeria* spp. and *Listeria monocytogenes* Occurrence in Apple and Stone Fruit Orchards

Ishani Sheth[1], Darina Kantsavenka[2], Hee jin Kwon[1], Dohee Kim[2], Jessica Palmer[2], Abby Gao[2], Anna Wooten[2], Kari Peter[3], Yi Chen[1] and **DUMITRU MACARISIN**[1]

[1]U.S. Food and Drug Administration, College Park, MD, [2]U.S. Food and Drug Administration-CFSAN, College Park, MD, [3]Department of Plant Pathology and Environmental Microbiology State Fruit Research and Extension Center, College Park, MD

Introduction: *Listeria monocytogenes* (*Lm*) outbreaks linked stone fruits and to whole apples, used in producing caramel apples, highlights the potential for these fruits to serve as a vehicle in *Lm* transmission. Trace-back investigations of fresh produce-related outbreaks often implicated the growing environment as the source of contamination.

Purpose: Examine the prevalence of generic *Listeria spp.* and *Lm* in preharvest production environments of apple and stone fruit.

Methods: Fruits (apple and peach), leaves, soil, and surface water samples were collected from orchards in PA from July to October 2016. Samples were collected from orchards of early, mid and late season cultivars of apples and peaches. *Listeria* detection was conducted by selective enrichment in buffered *Listeria* enrichment broth, followed by streaking on MOX, ALOA, and *RAPID'L.mono* agars after 24 and 48 h of enrichment at 30°C. Presumptive *Listeria* positives were confirmed using API *Listeria* (bioMérieux).

Results: Overall, 0.9% of the fruits (n=110) and 0.9% (n=207) of the preharvest environmental samples were positive for *Lm*. Major *Listeria spp.* present on fruits were *Listeria grayi* (0.9%) and *Listeria welshimeri* (1.8%). The incidence and prevalence of *Listeria* spp. in environmental samples was greater than that in fruits. Major *Listeria* spp. in environmental samples were *L. grayi* (1.9%), *Listeria innocua* (0.9%), *L. welshmeri* (0.9%), *Listeria seeligeri* (0.48%) and *Listeria ivanovii* (0.48%). *Lm* was not detected in the orchard pond.

Significance: The potential exists for *Lm* contamination to enter packing houses on fruit or leaves at a low prevalence rate or through crates that were in contact with soil. Obtaining the baseline surveillance data on the occurrence of *Listeria spp.* and *Lm* in pome and stone fruit orchards will facilitate the development of preventive control strategies to reduce future recalls/outbreaks associated with these commodities.

P1-41 Isolation of *Salmonella* and *Campylobacter* Strains from Superficial Irrigation Water, Soil, and Vegetables Samples from Small Agriculture Fields around the Capital of Chile

LISETTE LAPIERRE[1], Constanza Vergara[1], Patricio Retamal[1] and Maria Cristina Martinez[2]

[1]Universidad de Chile, Santiago, Chile, [2]Instituto de Salud Publica, Santiago, Chile

Introduction: In Chile and around the world, people prefer to buy their vegetables from farmers market and the possibility of these vegetables has been contaminated with pathogenic microorganisms constitutes a risk to human health. The isolation of these two pathogens in vegetables, irrigation water and soil where they are produced could explain that the contamination on the final product could come from its origin.

Purpose: Isolate *Salmonella* and *Campylobacter* in irrigation water samples, leafy vegetables, and agriculture soils in small farms on the vicinity of the capital city of Santiago.

Methods: Samples are taken every two months along one year in six different points surrounding Santiago city. These points previously indicated the presence of enteric *Salmonella* isolated from superficial irrigation water creek, genotypically related to isolates from human patients. The points are distributed in an agricultural production area of 145 hectares. The samples correspond to superficial irrigation water, soil, and leafy vegetables within a radius of five kilometers from the creek. All samples were analyzed using ISO 6579-2 for *Salmonella* and FDA BAM for *Campylobacter.*

Results: There has been no isolation from the superficial irrigation water samples. From the 130 samples of soil, only one was positive for *Salmonella spp*. In vegetables, from 650 samples only two were positive for *Salmonella* spp. and 21 for *Campylobacter* spp.

Significance: The consumption of raw leafy vegetables from small farmers is very frequent and the preference for this type of food is increasing all over the world. This study shows that must be considered as a potentially vehicle for the transmission of pathogenic bacteria to the population. Being able to assess the level of contamination in the small agricultural production area and establish the source from this contamination is important to be able to adopt the corresponding control measures at the farms markets.

P1-42 Flies as Possible Vectors for Transfer of Shiga-toxigenic

Stuart Gorman[1], **VALERIE NETTLES**[1], Dara Smith[1], David Paulsen[2], Rebecca Trout Fryxell[2], Annette Wszelaki[3], John Buchanan[4] and Faith Critzer[1]

[1]University of Tennessee, Department of Food Science, Knoxville, TN, [2]University of Tennessee, Department of Entomology and Plant Pathology, Knoxville, TN, [3]University of Tennessee, Department of Plant Sciences, Knoxville, TN, [4]University of Tennessee, Department of Biosystems Engineering and Soil Science, Knoxville, TN

Introduction: Cattle and wildlife are known reservoirs for Shiga-toxigenic *Escherichia coli* (STEC) and *Salmonella*. Cattle operations may be in close proximity to farms growing fresh produce, allowing insects to transfer these pathogens to the crops. The role of insect vectors in produce contamination is not fully understood, and data characterizing the prevalence of foodborne pathogens in farm fly populations is lacking.

Purpose: Investigate the prevalence of STEC and *Salmonella* associated with various genera of flies sampled/collected on a farm with both produce and beef cattle.

Methods: Flies were collected using ten separate bait traps spread throughout the farm ranging in distance from cattle to plots used for growing and harvesting fresh produce. Flies were identified and grouped by trap location, collection date, and genus/species. Pooled samples were preenriched, followed by selective enrichment of *Salmonella* and STEC, respectively. Selective enrichment for *Salmonella* was plated onto XLT4 and colonies were confirmed using immunological latex agglutination. Selective enrichment for STEC was plated onto Chromagar STEC and presumptive-positive colonies were confirmed using quantitative real-time PCR for *stx1*, *stx2*, and *eae* genes.

Results: Over eight weeks, 152 pooled samples were collected representing 26 genera from five taxonomic families. Seventeen samples were positive for the presence of *Salmonella* (11 *Muscidae*, one *Sarcophagidae*, two *Calliphoridae*, one *Tachinidae*). Four samples were positive for the presence of

Poster

STEC (three Muscidae, one Sarcophagidae). There was no significant correlation between trap location, capture date, and pathogen presence among samples.

Significance: Our findings suggest that certain genera of flies may be more likely to carry and transmit foodborne pathogens from fecal material to produce on-farm. Further research is needed to determine the impact of cattle density, produce type and farm size as well as interventions that may alter fly patterns to reduce the risk of produce contamination.

P1-43 Plant Growth-promoting *Pseudomonas* spp. Reduces the Persistence of *Salmonella* spp. on Spinach and Tomato Leaf Surfaces

CHIUN-KANG HSU and Shirley Micallef
University of Maryland, College Park, MD

Introduction: *Salmonella enterica* can associate with plants, effectively using them as alternative hosts in between animal infection. *Salmonella* persistence in produce fields may lead to salmonellosis outbreaks. Intervention strategies to reduce the association of *Salmonella* bacteria with produce at the pre-harvest stage are needed.

Purpose: Plant-growth promoting rhizobacteria (PGPR) associate with plant roots to boost plant growth and health. In this study, we assessed the effect of two PGPR strains of *Pseudomonas* colonizing spinach and tomato roots on epiphytic *Salmonella enterica* Newport.

Methods: Tomato (Moneymaker) and spinach (Tyee) were grown for four and six weeks, respectively, and inoculated with two PGPR strains of *Pseudomonas*, S2 and S4, provided by Dr. Brian Klubek. Root inoculation with PGPR was conducted by applying three ml of culture (>10^9 CFU/ml) of S2 or S4 to the growing seedlings, twice postgermination. Leaves of spinach and tomato were spot inoculated with 1 x 10^6 CFU *Salmonella* Newport adapted for rifampicin resistance on two leaves per plant and harvested after 24 hours for bacterial enumeration on Tryptic Soy Agar amended with 50 µg/ml rifampicin. All spinach and tomato plants were grown and tested under BSL-2 conditions held at a constant temperature and relative humidity.

Results: Spinach and tomato plants inoculated with *Pseudomonas* S2 or S4 resulted in a statistically significant increase in shoot dry biomass by an average of 50% over control plants (P<0.001). Spinach and tomato plants previously inoculated with *Pseudomonas* strain S4 maintained a smaller *Salmonella* Newport population on their leaves compared to the control (P<0.001). *Salmonella* Newport counts on treated and control spinach (Tyee) were measured at 4.6 and 5.9 log CFU/plant, and tomato (Moneymaker) at 4.7 and 5.3 log CFU/plant, respectively.

Significance: The survival of epiphytically associated *Salmonella* populations on plants may be impaired by plant-mediated effects induced by PGPR.

P1-44 Effect of Postharvest Cooling on the Microbial Quality and Storage of Florida Peaches

Jaysankar De, **BRUNA BERTOLDI**, Alan Gutierrez, Jubair Mohammad, Steven Sargent and Keith Schneider
University of Florida, Gainesville, FL

✦ ***Undergraduate Student Award Competitor***

Introduction: Peaches can be damaged by rough handling, especially when picked at their advanced (tree ripe) stages. Current room cooling procedure used by peach growers often result in delays up to >12 h, possibly compromising quality.

Purpose: Determine the efficacy of postharvest room cooling on microbial quality control during peach storage.

Methods: Peaches, harvested from different orchards in Florida, were collected from pallets (fresh), pre-pack (room-cooled), and post-pack line. Three trials with triplicate samples were analyzed (n=9). Each sample was a composite of five peaches (diameter ~ 6.5 cm) and was collected in sterile plastic bags, which were transported in ice-box to the lab and immediately analyzed. Hundred ml 0.1% sterilized peptone water was added to each bag, then aerobic plate counts (APC) and yeast and mold counts (YM) were determined. Peaches from the post-pack line were incubated at 1.1°C for 21 days. Representative samples were withdrawn on days 1, 7, 14, 21.

Results: The average APC from fresh samples, pre-pack and post-pack lines were all 5.2 log CFU/peach. At the end of the 21-day incubation period, the APC on peaches from the postpack line was 4.9 log CFU/peach. The average YM counts from fresh peach and room-cooled samples were 6.2 and 6.1 log CFU/peach, respectively. After packing, the YM counts on the postpack samples were 6.0 log CFU/peach and increased slightly to 6.2 log CFU/peach at the end of the 21-day incubation. The changes in microbial counts on peaches from different stages of the postharvest process were not statistically significant (P>0.05). The APC was significantly higher (P<0.005) in trial three as compared to the other two trials, possibly due to heavy rainfall prior to harvest.

Significance: Information obtained will be used to recommend the best temperature management for maintaining the postharvest quality of peaches.

P1-45 Comparison of Forced-air Cooling and Hydrocooling on the Microbial Quality Control of Florida Blueberries

JAYSANKAR DE[1], Aswathy Sreedharan[1], You Li[2], Alan Gutierrez[1], Steven Sargent[1] and Keith Schneider[1]
[1]University of Florida, Gainesville, FL, [2]Beijing Technology and Business University, Beijing, China

Introduction: Blueberries are easily damaged by rough handling and adverse temperatures. Cooling procedures used by blueberry growers often result in delays up to 24 h, compromising quality.

Purpose: Evaluation of forced-air cooling (FAC), and hydrocooling with (HCS) or without sanitizers (HC) for microbial quality control of blueberries.

Methods: A cocktail of five rifampicin-resistant *Salmonella enterica* strains (Newport, Javiana, Enteritidis, Typhimurium and Braenderup) was utilized. Freshly harvested blueberries (170 g in 6-oz clamshell per replicate) were spot-inoculated with 10 µl inoculum, resulting in seven log CFU/blueberry, and were left to dry for one hour. Four sets, each consisting of three clamshells, were used in the experiments. One set was placed under a modified forced-air cooling unit for 60 to 90 minutes. One set each was hydrocooled for six minutes (with agitation) by complete immersion in water i) containing 150 ppm HOCl sanitizer solution and ii) without sanitizer. After hydrocooling, drained clamshells were placed on paper towels and weighed. All sets, including a control set (inoculated but not treated) were stored at 2°C and *Salmonella* was enumerated on days 0, 1, 3, 5, 7, 14, and 21 after inoculation.

Results: The FAC showed no significant effect (P>0.05) on the microbial quality of blueberries. The HC alone reduced the initial number (5.7 log CFU/g blueberries) of *Salmonella* significantly (P<0.01) on day 0. This decrease was most probably due to the mechanical action of the hydrocooling water washing the inoculum off the surface of the blueberries. Hydrocooling with sanitizer highly significantly (P<0.0001) decreased the number of inoculated *Salmonella* by >4 log CFU/g blueberries over 21-days incubation.

Significance: Hydrocooling, with or without sanitizer, of blueberries shows promising result in postharvest microbial quality control and storage of the fruits. Information obtained will be used for recommending the best temperature management to maintain postharvest quality of blueberries.

P1-46 In Silico Evaluation of a Novel Iterative Bayesian Sampling Strategy for Efficient Detection of Pathogenic Bacteria in Preharvest Produce and Environments

AIXIA XU[1] and Robert Buchanan[2]

[1]Department of Nutrition and Food Science, University of Maryland, College Park, MD, [2]University of Maryland, Department of Nutrition and Food Science and Center for Food Safety and Security Systems, College Park, MD

❖ ***Developing Scientist Competitor***

Introduction: Sampling of preharvest environments and produce is, increasingly, being used as a tool to enhance food microbial safety. In most sampling plans, the sample locations are determined beforehand and all samples are collected at once. This, in part, is because traditional methods of microbial detection take one or more days to yield results. However, recent development of rapid microbial methods allow the users to get test results much faster, which makes iterative sampling strategy possible.

Purpose: The goal of this study was to evaluate the effectiveness of traditional sampling plans and a novel iterative sampling strategy, based on Bayesian Global Optimization (BGO), on simulated fields with realistic contamination sources.

Methods: The effectiveness of the iterative BGO sampling plan and three traditional sampling plans (random, stratified-random, and z-pattern) were evaluated using a simulation model. Preharvest fields with realistic contamination sources were generated in silico. Three types of contaminations were considered, point contamination, line contamination and planar contamination. It was assumed that pathogen presence was correlated with indicator bacteria level. The BGO plan uses prior results to inform the subsequent sampling locations, to maximize overall detection probability. The same number of samples was collected in each sampling plan (n=18).

Results: In simulated fields with five by six plots and nine subplots/plot (270 total sampling locations and six contamination sites on average), the BGO sampling plan dramatically increased detection probability compared to traditional sampling plans (random: 0.30±0.11; stratified random: 0.32±0.11; z-pattern: 0.32±0.17; BGO: 0.63±0.23). The difference was highly significant (P<0.0001).

Significance: This study provides a novel iterative sampling strategy for microbial quality testing. The sampling strategy gives much better detection probability than traditional sampling plans in realistic scenarios. This alternative sampling approach would be particularly beneficial when implemented as part of testing program that monitors preharvest fields over the course of the cultivation cycle.

P1-47 Survival of *Listeria monocytogenes* on the Surface of Basil, Cilantro, Dill, and Parsley Plants Grown in a Greenhouse Environment

Cameron Bardsley[1], **LAURA STRAWN**[2], Rachel Pfuntner[2], Laura Truitt[2], Renee Boyer[1] and Steve Rideout[2]

[1]Virginia Tech, Blacksburg, VA, [2]Virginia Tech - Eastern Shore AREC, Painter, VA

❖ ***Developing Scientist Competitor***

Introduction: Fresh herbs are popular ready-to-eat commodities, found in many dishes. *Salmonella* spp. and *Escherichia coli* O157:H7 survival have been observed on herbs grown in field and laboratory environments; however, little research has been performed on the survival of *Listeria monocytogenes* on herbs grown in greenhouse environments.

Purpose: The objective of this study was to evaluate the survival of *L. monocytogenes* on the surface of basil, cilantro, dill, and parsley plants grown in a greenhouse.

Methods: Basil, cilantro, dill, and parsley plants were grown in a greenhouse with average temperature of 25±8°C and relative humidity of 63±20%. Upon maturity, basil, cilantro, dill, and parsley plants were inoculated with a five-strain, nalidixic acid-resistant cocktail of *L. monocytogenes*. Samples were enumerated using standard methods at time-points: 0, 0.21, 1, 2, 3, 7, 14, 21, and 28 days. JMP was used to perform statistical analysis using Tukey's multiple comparison test with a P-value <0.05.

Results: *Listeria monocytogenes* populations demonstrated a similar biphasic survival curve on each of the four different herb plants. No significant difference was observed in *L. monocytogenes* survival over 28 days, for each of the four herb plants. *Listeria monocytogenes* populations decreased by ≥ three log CFU/g on each herb plant by seven days postinoculation. The largest *L. monocytogenes* population reduction (basil: 3.6 log CFU/g, cilantro: 2.2 log CFU/g, dill: 2.1 log CFU/g, and parsley: 2.3 log CFU/g) was observed between zero and one day. While *L. monocytogenes* did not grow on herbs plants grown in a greenhouse environment, *L. monocytogenes* was able to survive up to 28 days post-inoculation for all the herb plants, excluding parsley, which fell below the limit of detection on seven days.

Significance: Since *L. monocytogenes* exhibited long-term survival on the herb plants studied, the need for greenhouses to implement best practices (e.g., sanitation, good agricultural practices) is critical to minimize the introduction of contamination.

P1-48 Investigation of Microbial Contamination Sources during Production of Radish Sprout

SE-RI KIM, Hyun-mi An, Bohyun Yun, Won-Il Kim, Sanghyun Han, Hyun-Ju Kim, Byeong-Yong Park and Jae-Gee Ryu

Microbial Safety Team, Agro-Food Safety & Crop Protection Department, National Institution of Agricultural Science, Rural Development Administration, Wanju, South Korea

Introduction: In recent years, raw sprouts have been involved in a number of cases of foodborne illness. Processing stages, such as seed soaking, germination, sprouting and packing provide many opportunities for bacterial contamination.

Purpose: The purpose of this study was to investigate what source most contributes to contaminating radish sprout by assessing microbial loads on sprout and agricultural inputs that came into contact with the sprout.

Methods: To estimate microbial loads, fecal indicators (coliform and *Escherichia coli*) as well as foodborne pathogens (*E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*) were enumerated. A total of 180 samples including seeds, sprouts, hydroponic nutrient solution, irrigation water, and tools from three different radish sprout farms were tested.

Results: Coliform contamination observed from sprout cutting knife and germinating seeds for all farms. For *E. coli* contamination, 14.4% (26 of 180) of the samples were positive, of which mostly sprouts and irrigation water collected from one particular farm. This implies that irrigation water could have served in transmitting *E. coli*within the production environment to the sprout being grown in the same premises. No foodborne pathogen was detected from the sample tested.

Significance: This study suggests more cautions should be taken if water source for sprout production is contaminated with potential fecal matters or foodborne pathogens.

P1-49 Investigation of Fecal Contamination Indicators and Foodborne Pathogens for Irrigation Water Used in Napa Cabbage Cultivation

SE-RI KIM, Bohyun Yun, Hyun-mi An, Won-Il Kim, Sanghyun Han, Hyun-Ju Kim, Byeong-Yong Park and Jae-Gee Ryu
Microbial Safety Team, Agro-Food Safety & Crop Protection Department, National Institution of Agricultural Science, Rural Development Administration, Wanju, South Korea

Introduction: Numerous foodborne illness outbreaks associated with consumption of kimchi have been reported, in recent years. Irrigation water was considered as a major pathogenic bacteria contamination source of Napa cabbage used as an ingredient in kimchi.

Purpose: The purpose of this study was to investigate fecal indicators (coliforms and *Escherichia coli*), as well as foodborne pathogens *(E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogene*s) in irrigation water used for Napa cabbage cultivation.

Methods: A total of 111 samples, including stream water (*n*=60), groundwater (*n*=36), and water in ponds (*n*=15), were collected from five different regions of Korea where Napa cabbage is massively grown. Fecal indicators and foodborne pathogens in irrigation water were analyzed by biochemical methods.

Results: The numbers of coliform from stream water, water in ponds, and ground water were 1.96 to 4.96, 2.71 to 3.78, and 0 to 3.98 log MPN/100 ml, respectively. *Enterococci* were detected in 95% (57 of 60) of th stream water samples, 80% (12 of 15) of water from ponds, and 19% (7 of 36) of ground water samples and ranged from 0 to 4.00, from 0 to 2.00, and from 0 to 2.76 log MPN/100 ml, respectively. Additionally, 97% (58 of 60) of stream water, 93% (14 of 15) of water in ponds, and 22% (8 of 36) of ground water samples were observed being contaminated with *E. coli*. Only 0.9% (1 of 111) of the irrigation water samples was positive for *L. monocytogenes*.

Significance: Data from this study enables microbial risk assessment for irrigation water used in Napa cabbage cultivation.

P1-50 Recovery of *Salmonella* Agona and Typhimurium on Sprouting Alfalfa after Seed Sanitation

YUE DAI[1], Pascal Delaquis[2], Carmen Wakeling[3] and Siyun Wang[1]
[1]University of British Columbia, Vancouver, BC, Canada, [2]Agriculture and Agri-Food Canada, Summerland, BC, Canada, [3]Eatmore Sprouts & Greens Ltd., Courtenay, BC, Canada

Introduction: Alfalfa sprouts contaminated with *Salmonella* have been the source of many foodborne disease outbreaks in North America. Although treatments recommended by the Canadian Food Inspection Agency (CFIA) achieve a minimum three log reduction immediately after sanitation, previous research has shown that *Salmonella* can proliferate during germination. The recovery of *Salmonella* after sanitation is of great interest as it may explain the persistence on sprouted vegetables.

Purpose: The purpose of this study was to investigate the ability of *Salmonella* Agona and Typhimurium to grow on alfalfa sprouts after CFIA-recommended seed treatments and a treatment compliant with organic production principles.

Methods: Alfalfa seeds inoculated with *Salmonella* Agona PARC 5 isolated from alfalfa sprouts or *Salmonella* Typhimurium PARC 64 (4.67±0.26 log CFU/g) were subjected to three types of sanitizing treatments: 5,000 ppm chlorine, 8% hydrogen peroxide, and an organic treatment (50°C hot water, 2% hydrogen peroxide, and 0.1% acetic acid). The sanitized seeds were sprouted and *Salmonella* were recovered on xylose lysine deoxycholate agar during germination. Survival curves were plotted and compared using one-way ANOVA.

Results: The density of both *Salmonella* serovars increased from <10 CFU/g immediately after treatment to 5.5 to 7.5 log CFU/g after six days of germination. The lag phase of both serovars, after the chlorine treatment, were significantly shorter (P<0.05) compared to those after the other two treatments. The maximum growth rate of *Salmonella* Agona was greater (P<0.05) than Typhimurium. For instance, the cell density of *Salmonella* Agona reached 7.34±0.27 log CFU/g 24 hours after the chlorine treatment versus 5.77±0.51 and 6.87±0.14 log CFU/g after the hydrogen peroxide and the organic treatments, respectively.

Significance: These data show that *Salmonella* cells were able to recover and grow on sprouting alfalfa seeds. More lethal treatments need to be devised for production of pathogen-free sprouted vegetables.

P1-51 Sunlight Exposure Reduces Viability in *Salmonella enterica*

GOVINDARAJ DEV KUMAR[1], Shirley Micallef[1] and Dumitru Macarisin[2]
[1]University of Maryland, College Park, MD, [2]U.S. Food and Drug Administration, College Park, MD

Introduction: Adequate sunlight exposure is integral for crop health and yield, but can serve as a stressor for microorganisms.

Purpose: The effect of sunlight exposure on *Salmonella* culturability and survival was evaluated in PBS and on tomato fruit surfaces.

Methods: *Salmonella enterica* Newport expressing green fluorescent protein (GFP) was used to inoculate phosphate buffered saline (PBS) and tomato fruit (Heinz) surfaces. PBS aliquots (18 ml) or tomatoes were inoculated with 2 ml and 100 µl of inoculum, respectively, to a final concentration of 6.5 log CFU/ml/tube or /tomato. Inoculated fruit and PBS were exposed to sunlight from 11:30 AM to 5:30 PM (average temperature, 26.3°C; ultraviolet index, 5.16). Control samples were kept at the same temperature without light exposure. Samples were evaluated for culturable cell counts by plating on tryptic soy agar (TSA). GFP fluorescence in *Salmonella* Newport cells in PBS and tomato rinsates was measured as fluorescence units (FU) with a plate reader to assess bacterial viability. Imaging and live/dead staining was performed with laser scanning confocal microscopy.

Results: *Salmonella* Newport in PBS exposed to sunlight lost culturability, resulting in no detectable cells in culture, while the control PBS sample yielded 6.4±0.3 log CFU *Salmonella* Newport/ml (P<0.05). Fluorescence from viable *Salmonella* Newport cells suspended in PBS was 63.3±0.6 FU for test and 60.7±8.5 FU for control samples. Tomatoes not exposed to sunlight yielded 5.9±0.3 log CFU *Salmonella* Newport/tomato, while only 4 of 25 inoculated fruit exposed to sunlight yielded recoverable *Salmonella* Newport colonies on TSA; averaging 1.8±0.4 log CFU/tomato (P<0.05). FU from *Salmonella* Newport cells in tomato rinsate, from sunlight exposed tomatoes, was 43±1.0 and unexposed control tomatoes was 33±8.1 (P>0.05). Microscopy corroborated the presence of live *Salmonella* in all samples through both *gfp* expression and membrane permeable dyes.

Significance: Sunlight exposure may affect the recovery, but not viability of *Salmonella* cells.

P1-52 Metabolic Profiling of Non-O157:H7 Shiga Toxin-producing *Escherichia coli* Isolated from Spinach

GOVINDARAJ DEV KUMAR[1], Shirley Micallef[1] and Peter Feng[2]
[1]University of Maryland, College Park, MD, [2]U.S. Food and Drug Administration, College Park, MD

Introduction: Non-O157:H7 Shiga toxin producing *Escherichia coli* are a fast rising food safety threat resulting in the contamination of beef and produce.

Purpose: Sugar metabolism, amino acid metabolism and substrate utilization of 6 isolates of non-O157:H7 Shiga toxin producing *E. coli* isolated from spinach were assayed using a biochemical profiling strip and an enterobacterial profiling test strip.

Methods: Shiga toxin producing *E. coli* strains O181:H49, O91:H21, O113:H21, O98:H36, O116:H21, O11:H15 were tested for their indole, methyl red, Voges-Proskauer and citrate profiles. Carbohydrate metabolism traits were assayed for the following sugars: lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, melibiose, sucrose, L-arabinose, mannose, inulin, sodium gluconate, glycerol, salicin, dulcitol, inositol, sorbitol, mannitol, adonitol, arabitol, erythritol, alpha-methyl-D-glucoside, rhamnose, cellobiose, melezitose, alpha-methyl-D-mannoside, xylitol, ONPG, esculin, D-arabinose, citrate, malonate and sorbose. Lysine, ornithine, urease, nitrate and H_2S reactions were also assayed. These were performed by streaking test organisms on Tryptic Soy Agar plates and incubating at 37°C for 24 h. Suspension of isolates were prepared in Phosphate Buffer Saline (PBS) and inoculated into test ampules as per manufacturer's recommendation. Biochemical tests were conducted after 24 h incubation using appropriate reagents and protocol suggested by kit manufacturer.

Results: Differences in biochemical and amino acid metabolic profiles were observed between the isolates. *E. coli* strains O11:H15 and O91:H21 were negative for ornithine metabolism while all other isolates were positive. *E. coli* strain O116:H21 was positive for phenylalanine metabolism while all other isolates were negative. All isolates showed typical positive reactions for indole and methyl red and Voges-Proskauer tests. Differences in citrate and saccharose metabolism was observed between the isolates. *E. coli* O98:H36 was negative for raffinose metabolism while all other isolates were positive.

Significance: Difference in sugar, amino acid and citrate metabolism traits could confer differential abilities to persist in environmental and food matrices.

P1-53 My *Salmonella* is Longer Than Yours: Filamentous Cell Phenotype in Response to Stress

GOVINDARAJ DEV KUMAR[1], Shirley Micallef[1] and Dumitru Macarisin[2]

[1]University of Maryland, College Park, MD, [2]U.S. Food and Drug Administration, College Park, MD

Introduction: Filamentation is an elongated morphotype of *Salmonella* where septation is delayed during cell division.

Purpose: Two stressors, sunlight and antimicrobial exposure, were evaluated for their ability to trigger formation of filamentous cells.

Methods: Green fluorescent protein-expressing *Salmonella* Newport (five log CFU/ml) was grown in tryptic soy broth (TSB) and TSB+20 mM pelargonic acid, a compound found in plants and used as a herbicide. Incubation at 37°C was followed with enumeration and imaging using light and confocal microscopy at various time points. Phosphate buffer saline (PBS) was inoculated with *Salmonella* Newport to a concentration of 6.5 log CFU/ml and exposed to sunlight from 11:30 AM to 3:30 PM at an average temperature of 26.3°C and ultraviolet index of 5.16. Controls were incubated at the same temperature in the dark. Samples were subsequently imaged under a confocal microscope.

Results: Exposure to both sublethal concentration of pelargonic acid and sunlight resulted in two morphotypes–regular sized (5 µm) and filamentous (10 to 60 µm) cells. After 24 h, *Salmonella* Newport reached a culturable population of 8.6±0.2 and 3.5±0.9 log CFU/ml in TSB and TSB+20 mM pelargonic acid ($P<0.05$), respectively. Four hours of exposure to pelargonic acid was sufficient to initiate filamentation, with maximum filamentation observed at 16 h. By 24 h, a reduction in filamentous cells was observed, suggesting separation of cells. Sunlight exposure for four hours of *Salmonella* Newport-PBS suspension resulted in a loss of culturability of *Salmonella* Newport cells from an initial population of 6.5 log CFU/ml on TSA plates. PBS suspension consisted of both filamentous cells and regular sized cells. Both cell types expressed *gfp*, indicating viability. Adding aliquots of sunlight-exposed *Salmonella* Newport-PBS suspensions to TSB, followed by 24 h incubation, resulted in recovery of culturable cells.

Significance: *Salmonella* forms filamentous cells in response to stress, possibly as a stress mitigation strategy which may aid *Salmonella* survival in the agricultural environment.

P1-54 Isolation and Identification of *Listeria* spp., *Staphylococcus aureus,* and *Salmonella* during Dry Aging

HYEMIN OH, Jiyoung Lee and Yohan Yoon

Sookmyung Women's University, Seoul, South Korea

❖ ***Developing Scientist Competitor***

Introduction: Dry aging is one of the aging processes, especially for beef. Dry aging was used in the past, but it disappeared after the refrigerator was invented. This aging process is becoming popular because it produces flavor and texture, which consumers like. However, food safety concerns for the process have been raised.

Purpose: The objective of this study was to investigate the prevalence of *Listeria* spp., *Staphylococcus aureus*, and *Salmonella* in beef carcasses during dry aging.

Methods: Surface samples from 27 beef carcasses and 26 environmental samples (door knob, knife, tray, cutting board, shelve, and fans in aging room) in dry aging room by swabbing method with sterile gauze. Swab samples were then plated on Palcam agar (*Listeria* spp.), mannitol salt agar (*Staphylococcus aureus*), and xylose-lysine-desoxycholate agar (*Salmonella*). Typical colonies from each plate were further analyzed by 16S rRNA analysis and PCR for identification. To detect toxigenic genes in each isolates, PCR were performed using the primers specific to pathogenic genes.

Results: Of 27 carcass samples, *Listeria* spp. were isolated from three samples (11.1%), and two samples had *Listeria ivanovii* (7.4%) and one sample had *Listeria monocytogenes* (3.7%). *Staphylococcus aureus* was also isolated from 16 samples (59.3%), but there were no samples contaminated with *Salmonella*. Of 26 environmental samples, two samples (7.7%; one cutting board and one shelf) were contaminated with *Salmonella*. *Listeria monocytogenes* (3.9%) was, also, isolated from the cutting board. The *L. monocytogenes* isolates had pathogenic genes such as *hly*A and *prs*.

Significance: These results indicate that food safety criteria need to be established to improve food safety for dry aging beef.

P1-55 Inactivation of Norovirus during Smoked Salmon Storage

HYEMIN OH[1], Yewon Lee[1], Hyun Jung Kim[2], Changsun Choi[3] and Yohan Yoon[1]

[1]Sookmyung Women's University, Seoul, South Korea, [2]Korea Food Research Institute, Gyeonggi, South Korea, [3]Chung-Ang University, Ansung, Kyounggi, South Korea

❖ ***Developing Scientist Competitor***

Introduction: Human norovirus mainly caused gastroenteritis through intake of raw seafood and vegetables with a low infectious dose of 10 to 100 viral particles. Since the consumption of smoked salmon has been recently increased, the possibility for norovirus outbreak by smoked salmon has also increased.

Purpose: The objective of this study was to determine the survival of norovirus in smoked salmon using murine norovirus as a norovirus surrogate at low temperature.

Methods: Murine norovirus was inoculated into 25 g of smoked salmon samples at four log PFU/g, and the smoked salmon samples were stored aerobically at 4°C (three days) and 20°C (two days). Enumeration of murine norovirus on smoked salmon was conducted by plaque assay with RAW

264.7 cells. For the initial titer plaque assay, RAW 264.7 cells were seeded into six-well plates at a concentration of 5×10^5 viable cells in two ml of minimum essential media to each well. The Baranyi model was fitted to the quantitative data for murine norovirus to calculate death rate (log PFU/g/h).

Results: The results of initial titer plaque assay showed that inoculate virus counts were 4.09 log PFU/g. Murine norovirus in smoked salmon was rapidly inactivated less than 48 h at 20°C. Murine norovirus titer were decreased from 4.09 to 1.5 log PFU/g at 4°C for 72 h. Death rates ranged from -0.02 (4°C) to -0.06 log PFU/g/h (20°C).

Significance: The results indicate that norovirus can survive at cold temperature rather than high temperature. Therefore, low amounts of norovirus remained at cold temperature storage can be infected from smoked salmon to human.

P1-56 Microbiological Quality and Flavor Profile of Alkaline Fermented Bambara Groundnut Made into a Dawadawa-type African Food Condiment Using *Bacillus* Species Starter Cultures

GABRIEL AKANNI, Henriette de Kock and Elna Buys
University of Pretoria, Pretoria, South Africa

❖ ***Developing Scientist Competitor***

Introduction: Dawadawa, an African food condiment, is a soup flavoring agent and low-cost meat substitute. It is typically produced through alkaline fermentation (pH 6 to 9) of African locust bean; but, there is interest in using other substrates, such as the bambara groundnut. Production from bambara groundnut is limited, despite the legume's high potential as a substrate due to its high carbohydrate and protein contents. Fermentation is usually spontaneous; thus, food safety and flavor consistency is a concern. The microbial diversity of alkaline fermentation of African condiments indicated several *Bacillus* species as the predominant genera. Therefore, controlled fermentation, utilizing specific *Bacillus* species as starter cultures, may improve food safety and flavor consistency.

Purpose: This study was performed to identify *Bacillus* species starter cultures for the controlled alkaline fermentation of bambara groundnut into Dawadawa-type condiments; determine their ability to suppress pathogenic microbial contaminants; and to determine volatile flavor compounds produced by these *Bacillus* species.

Methods: DNA sequencing of the 16S rRNA and *gyr*A genes was performed to identify *Bacillus* species starter cultures. Microbial growth parameters (specific growth rate, antimicrobial production) and physicochemical properties were analyzed during alkaline fermentation. Flavor compound profiles were determined using GC×GC–TOF MS.

Results: Molecular typed strains *Bacillus subtilis* subsp. *subtilis*, *Bacillus amyloliquefaciens*, *Bacillus pumilus* and *Bacillus licheniformis* were identified as starter cultures for fermentation with bambara groundnut. *Bacillus amyloliquefaciens* and *B. pumilus* had the highest μ_{max} at 0.14 and 0.13, respectively, and final pH values of 8.53 and 8.36, respectively. All strains have the ability to inhibit the growth of pathogenic microorganisms with levels of *Enterobacteriaceae* ≤ 7.0×10^1 CFU/g; molds ≤ 3.1×10^2 CFU/g. Significant differences in flavor profiles were apparent amongst starter cultures, with differing levels of acids, ketones, and pyrazines.

Significance: *Bacillus* strains as starter cultures for the alkaline fermentation of African condiments suppressed pathogenic microbial contaminants; thus, enhancing product food safety and flavor consistency.

P1-57 Microbiological Map of Selected Caribbean Foods over the 11-year Period 2004 through 2014

ZOE GORDON, Andre Gordon and James Kerr
Technological Solutions Limited, Kingston, Jamaica

Introduction: The Caribbean is home to 39.1 million people, with a further 28.7 million stop-over visitors in 2015, all of whom expect to get safe wholesome food. Because of the limited published data on the safety of Caribbean foods, very little is known about the conformance with industry standards and safety of Caribbean foods.

Purpose: The purpose of this study was to map the microbiological profile of selected Caribbean foods over an eleven-year period, 2004 through 2014. Samples evaluated included dairy and meat products, beverages, produce, sauces, prepared meals, coleslaw, salads and seafood.

Methods: Total aerobic plate counts (TAPC), *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella sp.*, and *Clostridium perfringens,* where relevant*,* were determined for 28,527 food and environmental samples, as was the microbiological quality of the production environment in selected production facilities. The number of samples exceeding acceptable limits and those found to be presumptively positive for selected pathogens for samples assessed by traditional and AOAC-accredited rapid methods were determined. Organisms resulting in presumptive positive findings for *Salmonella* and *L. monocytogenes* determined by the Reveal and traditional methods were also identified.

Results: Of the samples surveyed, 27,357 (95.9%) were within acceptable limits for the parameters assessed while 1,173 samples (4.1%) were not. For TAPC, 726 (10.3% of samples assessed) exceeded their respective limits. Of samples assessed for *E. coli, Salmonella, and L. monocytogenes*, 154 (4.1%), 28 (1.5%) and 26 (1.2%), respectively, were positive. For samples assessed for *Salmonella* and *L. monocytogenes* by the Reveal method that were found to be negative, *Enterobacter spp.* and *Enterococcus sp.* and *L. innocua,* respectively, were the major organisms found.

Significance: Caribbean foods assessed over the period demonstrated a low level of contamination with pathogens and excellent compliance with globally accepted microbiological limits. For *Salmonella, L. monocytogenes* and *C. perfringens,* food samples showed increasing compliance over time.

P1-58 Synergistic Effect of Heat and Elevated Hydrostatic Pressure for Inactivation of *Listeria monocytogenes*

ABIMBOLA ALLISON, Eleonora Troyanovskaya, Shahid Chowdhury and Aliyar Fouladkhah
Public Health Microbiology Laboratory, Tennessee State University, Nashville, TN

❖ ***Developing Scientist Competitor***

Introduction: Recent epidemiological investigations, derived from CDC active surveillance data, indicates 99% of illnesses caused by *Listeria monocytogenes* are foodborne in nature, leading to hospitalizations in 94% of episodes, and are collectively responsible for an estimated 266 annual deaths of American adults.

Purpose: This recent study investigated effects of elevated hydrostatic pressure on cell reduction and inactivation rates of *L. monocytogenes* at 4 and 55°C.

Methods: Various times (zero to 10 minutes) and intensity levels (zero to 380 MPa) of elevated hydrostatic pressure were investigated for inactivation of *L. monocytogenes* inoculated into phosphate buffered saline with target population of 7.5 log CFU/ml. Temperature was monitored, and maintained at 4 or 55°C by a circulating water bath with a stainless steel water jacket surrounding the chamber. The experiment was conducted in two biologically independent repetitions, as blocking factors of a randomized complete block design, containing three repetitions per time/temperature/

pressure within each block. Experimental data was analyzed by the SAS GLM procedure using Tukey- and Dunnett-adjusted ANOVA. The inactivation K_{max} and *D*-values were calculated using best-fitted (maximum R^2) model obtained by GInaFiT software.

Results: At 380 MPa (zero to 10 minutes), a *D*-value of 2.81 min and inactivation K_{max} of 1.60±0.41/min were observed at 4°C. At 55°C, these values were 1.59 and 3.94±0.96, respectively. At 4°C, the pathogen was reduced ($P<0.05$) by 3.84, 2.44, and 1.05 log CFU/ml after exposure to 10 minutes of hydrostatic pressure at 380, 310, and 240 MPa, respectively. These reductions ($P<0.05$) were >7.13, 6.36, and 4.53 for 10-minute treatments at 55°C, respectively. Treatments below two minutes were less efficacious ($P\geq0.05$) against the pathogen in the vast majority of the tested time, temperature, and pressure combinations.

Significance: Results of this study could be incorporated as part of a risk assessment modeling and predictive microbiology for reducing the public health burden of listeriosis.

P1-59 *Listeria* Interspecies Competition during Selective Enrichment Compared Using Three Regulatory Methods

Kaitlin Cauchon[1], Anthony Hitchins[2] and **RONALD SMILEY**[3]

[1]U.S. Food and Drug Administration, Jefferson, AR, [2]U.S. Food and Drug Administration (retired), Rockville, MD, [3]U.S. Food and Drug Administration/ORA/Arkansas Regional Laboratory, Jefferson, AR

Introduction: Selective enrichment is used to aid the recovery of *Listeria monocytogenes* from food samples. Resident microorganisms on the test product, not inhibited by the selective conditions, can hinder growth of *L. monocytogenes* and complicate its recovery, particularly when additional species of *Listeria* are present.

Purpose: This study evaluates the level of inter-species competition between *L. monocytogenes* and *Listeria innocua* during selective enrichment of spiked mung bean sprouts, using three regulatory test methods.

Methods: Portions of mung bean sprouts were spiked (one to five CFU/g) with *L. monocytogenes* (*n*=10 strains total) and *L. innocua* (*n*=1 strain). Selective enrichment was performed using the FDA BAM, the USDA-FSIS, or the EN ISO 11290.1 method. Quantitave PCR (qPCR) was used to enumerate *L. monocytogenes* in the double-species spiked sprout enrichments. *Listeria innocua* was enumerated using PALCAM agar.

Results: Following the selective enrichment of spiked mung bean sprouts, the *L. innocua*/*L. monocytogenes* population differentials were 2.8±1.1, 3.3±1.3, and 3.6±1.4 Δ log CFU/ml for the FDA BAM, USDA-FSIS, and EN ISO 11290-1 methods, respectively. In matrix-free enrichments, the population differentials were 1.7±0.9, 3.4±1.2, 2.1±1.2 Δ log CFU/ml for the same three methods, respectively. Considerable strain dependent variation was observed for *L. monocytogenes* with all three enrichment methods. The populations of *L. innocua* was always greater than *L. monocytogenes*.

Significance: The resident microorganisms on the sprouts contributed to the final population differentials observed between *L. innocua* and *L. monocytogenes* in both the FDA BAM and EN ISO 11290-1 methods. Resident microorganisms may negatively impact recovery of *L. monocytogenes* from high microbial load foods, following selective enrichment when multiple *Listeria* species are present. Continued improvement of selective enrichment for *L. monocytogenes* is needed to enhance the capabilities of regulatory laboratories to recover this organism amidst a complex microflora.

P1-60 Biofilm Formation and Sanitizer Resistance of *Listeria monocytogenes* in Mono- and Mixed-species with Cultivable Indigenous Microorganisms in Fresh Salmon

XINYI PANG[1], Chun Hong Wong[1], Hyun-Jung Chung[2] and Hyun-Gyun Yuk[3]

[1]National University of Singapore, Singapore, Singapore, [2]Inha University, Incheon, South Korea, [3]Korea National University of Transportation, Chungju-si, South Korea

Introduction: *Listeria monocytogenes* is capable of adhering and forming biofilms on food contact surfaces for long-term persistence in food plants. In food processing environment, *L. monocytogenes* may coexist and interact with multiple species, with impact on both the biofilm structure and its response to sanitizer treatment.

Purpose: The objective of this study was to evaluate biofilm formation and sanitizer resistance of *L. monocytogenes* in mono- and mixed-species biofilms with cultivable indigenous microorganisms (CIM) from fresh salmon under simulated salmon processing environment.

Methods: Biofilms of *L. monocytogenes* in mono- and mixed-species with CIM from fresh salmon were formed on stainless steel coupons by incubating in diluted TSB (1:20) or salmon juice at 4 and 15°C, respectively. The biofilm density and sanitizer (Ecolab® Whisper™ V, quaternary ammonium compounds-based) resistance of *L. monocytogenes* were determined on day 1, 4, 7, and 14. Confocal laser scanning microscope (CLSM) was used to characterize the spatial structure of biofilms.

Results: Higher temperature and longer biofilm age improved biofilm formation by *L. monocytogenes.* Compared to diluted TSB, higher biofilm density of *L. monocytogenes* was observed in salmon juice. Biofilm density of *L. monocytogenes* was significantly ($P<0.05$) lower in mixed-species (6.06 log CFU/cm^2) than single-species (5.29 log CFU/cm^2) at 15°C from day 4 to day 14, in salmon juice. However, *L. monocytogenes* biofilm cells in mixed-species were more resistant than those in single-species, with 0.42 and 2.85 log CFU/cm^2 log reductions on day 4, respectively. The honeycomb-like cell clusters shielding the bottom layer structure of biofilms shown by CLSM images might explain the enhanced sanitizer resistance of *L. monocytogenes* in mixed-species.

Significance: This study provides insights into *L. monocytogenes* biofilm formation under simulated salmon processing environment and highlighs that the presence of indigenous microorganisms could protect *L. monocytogenes* biofilm cells from sanitizer treatment.

P1-61 Cold Shock Domain Family Proteins Contribute to Virulence, Cellular Aggregation, and Flagella-based Motility in *Listeria monocytogenes*

Athmanya Eshwar[1], Roger Stephan[2] and **TAURAI TASARA**[2]

[1]Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland, [2]University of Zurich, Zurich, Switzerland

Introduction: The gram-positive bacterium *Listeria monocytogenes* is an important foodborne pathogen that causes listeriosis and high rates of mortality amongst those with weakened immunity. Cold shock domain family proteins (Csps) are small global gene expression regulating proteins implicated in stress protection and virulence responses in bacteria. *Listeria monocytogenes* harbors three Csps; CspA, CspB and CspD.

Purpose: This study examined Csps roles in *L. monocytogenes* virulence, cell aggregation, and motility phenotypes.

Methods: A wild type strain and a series of *csp* gene (Δ*cspABD*, Δ*cspBD*, Δ*cspAD*, and Δ*cspAB*) deletion mutants of *L. monocytogenes* EGDe were compared with respect to virulence, aggregation, and flagella-based motility.

Results: Without Csps, the capability of *L. monocytogenes* to survive inside human macrophages and induce virulence, in zebra fish, was severely diminished. Moreover, cellular aggregation, surface flagellation, and swarming motility was attenuated upon loss of Csps. Protein and mRNA based

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comparison of gene expression showed that the optimal expression of important virulence (*prfA*, *hly*, *mpl*, *actA*, and *plcB*) and flagella (*flaA*) associated genes in *L. monocytogenes*, also, depends on intact Csp functions.

Significance: Our studies shows that Csp-dependent regulation of the expression of key virulence and flagella genes plays an important role in enabling the optimal expression of host virulence, cellular aggregation, and flagella-based motility in *L. monocytogenes*.

P1-62 Diversity of *Listeria monocytogenes* Isolated from Clinical Cases and Food in Chile

Viviana Toledo[1], Henk Den Bakker[2], Marc Allard[3], Martin Wiedmann[4], Helia Bello[5], Gerardo Gonzalez-Rocha[5] and **ANDREA MORENO SWITT**[1]

[1]Universidad Andrés Bello, Santiago, Chile, [2]Texas Tech University, Lubbock, TX, [3]U.S. Food and Drug Administration, College Park, MD, [4]Cornell University, Ithaca, NY, [5]Universidad de Concepción, Concepción, Chile

❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* is an important foodborne pathogen that causes severe infections with high mortality rate. This bacterium is ubiquitously distributed in the environment and it can survive and grow in stress conditions as acid, salt, and low temperature; which are conditions usually found in food processing. In addition, a considerable variability in the capacity to cause human infection have been reported for *L. monocytogenes* lineages. Two listeriosis outbreaks and an increase on listeriosis incidence have been reported in Chile.

Purpose: The aim of this study was to phenotypically and genetically characterize *L. monocytogenes* isolated from human cases and foods in Chile.

Methods: A total of 37 isolates from different sources were selected for sequencing. Genomes were sequenced with Illumina and reads were assembled de novo using SPAdes. Isolates diversity was assessed with in silico MLST and serotyping. A maximum likelihood phylogenetic tree based on core genome SNPs was conducted. Phenotypic assays on ten isolates included determination of i) cold growth, ii) acid survival, iii) survival on oxidative stress, iv) growth in saline solution, and v) Caco-2 cells invasion. At least three biological replicates were performed and ANOVA test was used for statistical analysis.

Results: The phylogenetic analysis clustered Chilean isolates in two main lineages (I and II). MLST showed that worldwide-distributed clonal groups (CC-1 and CC-9) are also causing clinical cases in Chile. Among the isolates tested large phenotypic assays diversity was observed. No significant differences between lineages were found, except for salt stress assay, in which lineage I isolates showed a better ability to survive at higher concentrations of salt 1.65±0.14 versus 1.52±0.23 CFU/ml from lineage II ($P<0.05$).

Significance: This study provides baseline data on the genomic and phenotypic diversity of *Listeria* circulating in Chile and further supports global distribution of key human disease associated *L. monocytogenes* clonal groups.

P1-63 Extended Exposure to Low-temperature Stress Promotes the Formation of *Listeria monocytogenes* variants with Enhanced Cold, Acid, and Salt Tolerance

PATRICIA HINGSTON[1], Lisbeth Truelstrup Hansen[2] and Siyun Wang[1]

[1]University of British Columbia, Vancouver, BC, Canada, [2]Technical University of Denmark, Kgs. Lyngby, Denmark

❖ ***Developing Scientist Competitor***

Introduction: The human pathogen *Listeria monocytogenes* (*Lm*), is commonly recognized for its ability to contaminate foods and grow during refrigerated storage. However, some strains are more cold tolerant than others. Given the importance of preventing *Lm* from reaching unsafe levels in food, little is known about conditions that select for the evolution of more cold tolerant strains.

Purpose: The aim of this study was to determine if prolonged cold stress exposure would promote the formation of *Lm* variants with enhanced cold tolerance and/or resistance to other food-related stresses.

Methods: BHI cultures of 11 *Lm* strains were grown to stationary phase at 4°C and then maintained at this temperature for one year. Survivors were enumerated monthly and screened for the presence of variants with enhanced cold tolerance. Additionally, the pH and membrane lipid compositions of the long-term cold storage strains were compared to those of the parent strains. Isolates demonstrating enhanced cold tolerance were also subsequently evaluated for their salt (BHI+6% NaCl, 25°C) and acid tolerance (BHI pH 5, 25°C).

Results: Ten *Lm* variants with enhanced cold tolerance were successfully isolated from one of the 11 strains after 84 days of storage. At 4°C, the parent strain had a maximum cell density of 7.21±0.10 log CFU/ml while the variants reached 8.96±0.02 CFU/ml. Similarly, the parent strain had a maximum growth rate (μ_{max}) of 0.58±0.03 log CFU/ml/h while the variants had a μ_{max} of 0.94±0.02. Two of these variants also exhibited significantly ($P<0.05$) increased salt and acid tolerance. After one year of storage the pH of the cultures decreased from 7.10±0.03 to 5.68±0.03 and the number of survivors ranged from 5.70 to 7.51 log CFU/ml.

Significance: Our results show that prolonged cold stress exposure either in an environmental or food processing setting can promote the development of *Lm* variants with enhanced tolerances to food-related stresses.

P1-64 Changes in Zones of Inhibition and Minimum Inhibitory Concentrations of Antibiotics in *Listeria Monocytogenes* Strains after Exposure to Chlorine-induced Sublethal Oxidative Stress

Mohit Bansal, **RAMAKRISHNA NANNAPANENI** and Chander Shekhar Sharma

Mississippi State University, Mississippi State, MS

Introduction: When exposed to sublethal stresses, *Listeria monocytogenes* (*Lm*) may induce adaptive response to adverse stress conditions.

Purpose: The objective of this study was to determine the effect of sublethal oxidative stress induced by chlorine (sodium hypochlorite) against homologous stress and heterologous stress in two *Lm* strains, Scott A and V7.

Methods: *Lm* cells were exposed to increasing subinhibitory concentrations (20 ppm/day) of total chlorine from 250 to 375 ppm in tryptic soy broth (TSB). Changes in minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Lm* cells preexposed to chlorine at 250 (1/2 MIC), 330 (2/3 MIC) and 375 (3/4 MIC) and control (nonexposed to chlorine) were determined by macro-dilution method. Such chlorine-adapted *Lm* cells were evaluated for changes in Kirby-Bauer disk diffusion assay against 11 antibiotics (amoxicillin and clavulanic acid, ampicillin, ceftriaxone, streptomycin, nalidixic acid, gentamicin, sulphamethoxazole and trimethoprim, ciprofloxacin, rifampin, vancomycin). Also, chlorine-adapted and nonadapted *Lm* cells were evaluated for changes in MIC values to four antibiotics (ampicillin, gentamicin, streptomycin and tetracycline) as per CLSI guidelines.

Results: The MIC and MBC values of chlorine for *Lm* Scott A and V7 strains when exposed to sublethal chlorine were higher (600 ppm and 700 ppm) as compared to control (500 pm and 600 ppm). The average zones of inhibition by disk-diffusion assay for chlorine-adapted *Lm* cells was decreased by

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0.5 to 2.2 mm compared to control cells against all 11 antibiotics tested. Also, MIC values doubled for all four antibiotics tested against chlorine-adapted *Lm* cells compared to control in both strains. However, the changes in zones of inhibition and MIC values to all antibiotics tested for the chlorine-adapted and nonadapted control *Lm* cells were still under the susceptible range.

Significance: These findings indicate that the continuous exposure of *Lm* cells to chlorine may induce changes in homologues and heterologous stress adaptation.

P1-65 Antimicrobial Hydrogel Composed of Whey Protein or Maillard Reaction Products to Control Foodborne Pathogens

SEJEONG KIM, Hyemin Oh and Yohan Yoon
Sookmyung Women's University, Seoul, South Korea

❖ ***Developing Scientist Competitor***

Introduction: Heating, spraying, and dipping food in to various antimicrobials have been used to control foodborne pathogens in foods. However, these methods may not be appropriate to be used for certain foods. Thus, alternative decontamination methods need to be developed. Whey protein and maillard reaction products are byproducts from dairy industry, and they become gel-like form by chemical reaction.

Purpose: This study aimed at developing antimicrobial hydrogels which were made from whey proteins or its Maillard reaction products for food application.

Methods: Whey protein powder (1 to 5%) or Maillard reaction products (1 to 5%) from the reaction of hydrolyzed whey protein with sugars were mixed with water/dimethylsulfoxide (DMSO) solution at 1:0, 1:1, 3:7, 7:3, and 0:1 ratios. The mixtures were heated at 55°C overnight to precipitate proteins. Formed hydrogels were examined for properties (swelling, hardness, and elasticity) and antimicrobial loading capacity for grapefruit seed extract, citrus peel extract, and ε-polylysine. Eventually, the antimicrobial activity of the gels were evaluated to *Listeria monocytogenes*, *Escherichia coli*, *Salmonella*, and *Bacillus cereus*.

Results: Among combination of whey protein or Maillard reaction product concentrations with various ratios of water/DMSO, 3% whey protein with 0:1 ratio of water/DMSO and 3% Maillard reaction product with 0:1 ratio of water/DMSO had the most appropriate swelling, hardness, and elasticity. Regarding antimicrobial loading capacity, ε-polylysine was appropriate. Therefore, antimicrobial hydrogels (3% whey protein + 0:1 ratio of water/DMSO solution + ε-polylysine and 3% Maillard reaction product + 0:1 ratio of water/DMSO solution + ε-polylysine) were prepared and they showed antimicrobial activity against *L. monocytogenes*.

Significance: This result indicates that the developed antimicrobial hydrogels can be used to control *L. monocytogenes* in foods.

P1-66 Comparison of *Listeria* Swabbing Methods Using Residual Bacterial Method

NICOLE FAMILIARI, Delia Calderon and Paul Meighan
Hygiena, Camarillo, CA

Introduction: To test swabbing efficiency a superior method is to measure what was left behind on a surface rather than attempt to measure recovery from swabs. The binding of material or bacteria onto swabs can bias results of the number picked up.

Purpose: To test how effectively a range of Hygiena swabs recover viable dried bacteria from surfaces in comparison to routinely used standard environmental contact plates.

Methods: One hundred µl of *Listeria monocytogenes* dilutions (Dil -1, -2 and -3) were pipetted onto sterile coupons. Dilutions were dried on coupons measuring 4" by 4" and 12" by 12". The surface was left to dry for 24 h until visibly dry. Contact plates and three swab types (Dacron bud, small foam and large foam (InSite) swabs) were used to collect the dried bacteria from the surface and swabs were moistened with 100 to 400 µl of MRD depending on swab size. After sample collection, the coupons were put in whirl pack bags, 50 ml of MRD was added to the bag, each coupon was then mixed thoroughly to remove remaining bacteria. Each run used five replicate coupons both control and both tests (swab and contact plate). The unswabbed counts from the controls are considered 100% for comparison.

Results: The comparison of swabbing with any swabs proved that the pick-up was superior to the contact plates. The 4" by 4" coupons swabbed with Dacron bud swabs, small foam and large foam removed mean 91±6% (4" by 4"), 98±1% (4" by 4") and 91±3% (12" by 12") compared to contact plates which removed 72±16%, 71±7% and 50±12%. Comparing 4" by 4" to 12" by 12" coupons reduced the efficiency slightly but the pick-up was still superior to contact plates for *Listeria*.

Significance: Swabbing efficiency is better measured by using the residual bacteria method with proper controls.

P1-67 A Shelf-life Estimation and Growth of *Listeria monocytogenes on* Thawed Catfish Stored at Refrigerated Temperature

Thao. T.H. Nguyen[1], **VIJAY SINGH CHHETRI**[2], Karuna Kharel[2] and Achyut Adhikari[2]
[1]Research Center for Aquafeed Nutrition and Fishery Post Harvest Technology, Ho Chi Minh, Viet Nam, [2]Louisiana State University AgCenter, Baton Rouge, LA

Introduction: Recent outbreaks of *Listeria monocytogenes* with refrigerated foods highlighted a critical food safety issue associated with thawed catfish. At retail, frozen catfish after thawing are commonly sold and stored at refrigeration temperature for several days.

Purpose: This study examined the growth of *L. monocytogenes* and evaluated the shelf life based on aerobic plate count, yeast and mold counts on thawed catfish stored at refrigerated temperature.

Methods: Frozen catfish purchased from a local market was thawed and cut in 20 g pieces. *Listeria monocytogenes* (Strain 101M, serotype 4b) was inoculated on half of the samples and other half was used for aerobic plate count, and yeast and mold counts. Samples were stored in LDPE bags or petri plates at refrigerated temperature and microbiological examination was performed at every 24 hours for up to 144 hours.

Results: In both packaging conditions, there were no significant differences ($P<0.05$) in *Listeria* counts over the sampling period. After six days, the *Listeria* count increased by 0.70 log CFU/g (petri plate) and 1.06 log CFU/g (plastic bag). A significant increase in both aerobic plate count (4.12 to 7.90 log CFU/g) and yeast and mold count (3.21 to 5.63 log cfu/g) was observed within four days for the LDPE packed fish samples, however, sample stored on petri plates were less than 4.9 log CFU/g for both organisms. After six days, there was a significant increase in both aerobic plate count (to 8.79 log CFU/g and 6.63 log CFU/g) and yeast and mold count (to 6.10 log CFU/g and 4.31 log CFU/g) for LDPE packed and petri plates samples respectively.

Significance: These results confirm that *L. monocytogenes* can grow on the thawed catfish at refrigerator temperatures and indicate the importance of sanitation during thawing and storage. Our study validated that thawed catfish should not be stored more than four days in this condition.

Poster

P1-68 Presence and Distribution of *Listeria monocytogenes* in South African Meat and Meat Products

ITUMELENG MATLE[1], Evelyn Madoroba[1] and Khanyisile Mbatha[2]
[1]*Agricultural Research Council - Bacteriology Division, Pretoria, South Africa,* [2]*University of South Africa, Pretoria, South Africa*

❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* is an intracellular bacterium that is ranked as the third most serious cause of foodborne disease in the world. In South Africa, there is dearth of information about this pathogen and there are no publications about the extent of contamination of imported meat into South Africa.

Purpose: The aim of this study was to determine the presence of *L. monocytogenes* in abattoir, meat processing plants, and retail meat samples across nine provinces of South Africa and in imported meat samples.

Methods: A total of 2,013 of raw meat, meat products and ready-to-eat meat products were characterized using *L. monocytogenes* MicroSEQ(R)RT-PCR and classical microbiological techniques.

Results: The overall presence of *L. monocytogenes* was 10.9% (220 of 2,013). Out of 220 positive samples 85.5% (188 of 220) were from South Africa, while 14.5% (32 of 220) where from imported samples. Geographical results revealed that samples from Gauteng (34.0%; 64 of 188) North West (14.4%; 27 of 188) and Mpumalanga (14.4%; 27 of 188) provinces had the highest presence of *L. monocytogenes*, while Eastern Cape had a lowest occurrence (2.1%; 4 of 188). The samples collected from retail outlets were found to be the most contaminated (56.4%; 106 of 188), followed by the meat processing plants (38.8%; 73 of 188) and abattoir (4.8%; 9 of 188). A survey of various meat types showed that 59.0% (111 of 188) of processed meat samples had the highest presence of *L. monocytogenes*, followed by raw meat samples (25.5%; 25 of 188) and RTE meat products (15.4%; 29 of 188).

Significance: The presence of *L. monocytogenes* in various meat products in South Africa may pose a risk for human health. Therefore, the present research provided useful baseline information that will help in the development of policies and regulations for monitoring of *L. monocytogenes* in meat products in South Africa.

P1-69 Hygienic Design Shortcomings of Batch Frozen Dessert Freezers: Potential for Survival of *Listeria monocytogenes* in Ice Cream Mix-based Soil

Aisha Inuwa, Ashley Lunt, Brett Andersen, Davin Marta, **CHARLES CZUPRYNSKI** and Scott Rankin
University of Wisconsin - Madison, Madison, WI

Introduction: Although pasteurization and low temperature storage are interventions that make frozen dairy foods relatively safe, recent ice cream outbreaks raise concerns. One issue is potential design flaws (compared to NSF and 3A standards) in equipment used to produce frozen dairy desserts that might provide niches for *Listeria monocytogenes*. If this occurs, it could result in contamination of product.

Purpose: The overall purpose of this study was to evaluate design of equipment used to produce frozen dairy desserts for characteristics that might limit cleanability and allow survival of *L. monocytogenes*. We also evaluated the adherence, survival and resistance to sanitizers of *L. monocytogenes* in contaminated ice cream allowed to dry on stainless steel.

Methods: An expert on 3A and NSF sanitary standards examined equipment and highlighted several design issues that might result in difficult to clean niches. We also conducted experiments in which ice cream (5%, 10%, or 12% fat) was inoculated with a cocktail of *L. monocytogenes* strains, allowed to dry on stainless steel coupons and evaluated for *L. monocytogenes* survival and resistance to acid or chlorine-based sanitizers.

Results: We found sanitary design flaws such as rough finishes and surface irregularities that do not meet 3A or NSF standards and make these surfaces more difficult to efficiently clean and sanitize. When *L. monocytogenes* contaminated ice cream was allowed to dry on stainless steel coupons at 4°C, numbers of CFU remained at 7 log CFU for at least 12 weeks. Sanitizers had no detrimental effect on *L. monocytogenes* when used at a concentration and contact time (one minute) recommended by the manufacturer.

Significance: We find evidence that equipment used for frozen dairy desserts has design issues that might impair sanitization and provide harborage for *L. monocytogenes*. This is of potential concern because *L. monocytogenes* survives for extended periods in ice cream based soil, where it is more resistant to sanitizers.

P1-70 Characterization of *Vibrio parahaemolyticus* and *Vibrio vulnificus* Recovered from Oysters during the Salinity Relaying Process

Sara Elmahdi[1], Sylvia Ossai[1], Ligia DaSilva[1], John Jacobs[2], Michael Jahncke[3] and **SALINA PARVEEN**[1]
[1]*University of Maryland Eastern Shore, Princess Anne, MD,* [2]*NOAA, Oxford, MD,* [3]*Virginia Polytechnic Institute and State University, Blacksburg, VA*

Introduction: *Vibrio parahaemolyticus* (*Vp*) and *Vibrio vulnificus* (*Vv*) are naturally occurring estuarine bacteria and are the leading causes of seafood-associated infections and mortality in the United States of America. Though multiple-antibiotic resistant *Vp* and *Vv* have been reported due to misuse of antibiotics to control infections in aquaculture production, resistance patterns in vibrios are not as well documented as other foodborne bacterial pathogens. Moreover, information is limited regarding the influence of post-harvest treatment strategies on the phenotypic and genotypic characteristics of these pathogens.

Purpose: The purpose of this study was to evaluate the antimicrobial susceptibility, as well as the pathogenicity and genetic profiles of *Vp* and *Vv* recovered from oysters during the salinity relaying process.

Methods: *Vv* (*n*=296) and *Vp* (*n*=94) isolates were recovered from oysters, before and during the 21 day relaying study to detect virulence genes (*tdh*, *trh, vcgC*), using multiplex q-PCR. Antibiotic susceptibility to 20 different antibiotics was investigated using micro broth dilution and pulsed-field gel electrophoresis (PFGE) was used to study the genetic profiles of these pathogens.

Results: Twenty percent of *Vv* isolates were *vcgC*+, while 1 and 2% of *Vp* were *tdh*+ and *trh*+, respectively. More than 77% of *Vv* and 25% of *Vp* isolates were resistant to at least one antimicrobial. Forty eight percent of *Vv* and 8% of *Vp* isolates were resistant to two or more antimicrobials. All isolates demonstrated a high genetic diversity, even among those isolated from the same site and having a similar antimicrobial susceptibility profile. The relaying process had no significant effect on the antimicrobial susceptibility and PFGE profiles of *Vv* and *Vp* were observed (*P*=0.23).

Significance: This study provides a valuable information for risk management decisions, for both the oyster industry and regulatory agencies, as multiple antibiotic resistance represents a major concern in fish and shellfish farming and in human health.

Poster

P1-71 Virulence Assessment of *Vibrio* spp. in a *Galleria mellonella* Model

MIRA RAKIC MARTINEZ[1], Rohinee Paranjpye[2], Christopher Grim[1] and Atin Datta[1]
[1]*U.S. Food and Drug Administration, Laurel, MD,* [2]*NOAA, Northwest Fisheries Science Center, Seattle, WA*

Introduction: The increasing role of aquaculture in global food production highlights the importance for assessment of the virulence and pathogenicity of *Vibrio* spp.

Purpose: We evaluated different virulence factors and pathogenicity of *Vibrio anguillarum* and *Vibrio parahaemoliticus* in the greater wax moth *Galleria mellonella* animal model.

Methods: Eight strains of *V. anguillarum*, the causative agent of vibriosis in fish, and thirty strains of the seafood-borne human pathogen, *V. parahaemoliticus,* were evaluated. Wild-type and mutant strains of virulence genes: *vah1* and *rtxA* (*V. anguillarum*) and *pilA*, *mshA* and *gbpA* (*V.parahaemolyticus*) were also tested in *Galleria* model. Larvae were inoculated with individual *Vibrio* strains at concentrations of 10^6, 10^5, and 10^4 CFU/larva, monitored for mortality, (LT_{50} - time taken to kill 50% of insects), and for phenotype changes over seven days at 20°C.

Results: Differences in mortality were dose-dependent. Comparison between *Vibrio* spp. suggests a higher virulence associated with *V. parahaemolyticus* strains; an LT_{50} of 24 hours (dose of 10^6 CFU/larva) was observed for the majority of tested strains. LT_{50} of 48 hours was recorded for only three out of eight *V. anguillarum* strains. Insertions and in-frame deletion of *vah1* and *rtxA* genes of *V. anguillarum* resulted in reduced mortality in larvae. Natural isolates of *V. parahaemolyticus* from oysters, water, and planktons demonstrated higher virulence than clinical isolates, especially at the lower doses. The difference was strain-related and not statistically significant. The virulence of the wild-type *V. parahaemolyticus* measured by LT_{50} did not significantly differ from the isogenic *pilA*, *mshA* and *gbpA* deletion mutants at the 10^6 CFU/larva. At the lower doses, virulence of the wild-type strain was significantly (P<0.05) higher from D *pilA* and D *gbpA,* while D *mshA* did not differ.

Significance: Comparison between *Vibrio* spp. suggests a higher virulence potential associated with *V. parahaemolyticus* strains relative to *V. anguillarum* strains.

P1-72 Fitness of *Vibrio parahaemolyticus* in Seawaters at Different Oyster Harvesting Temperatures

CHAO LIAO and Luxin Wang
Auburn University, Auburn, AL

❖ ***Developing Scientist Competitor***

Introduction: *Vibrio parahaemolyticus* is a foodborne pathogen that presents ubiquitously in seawaters. In the United States, *Vibrio* illnesses were commonly associated with consuming raw or contaminated oysters. Over 50% of outbreaks of *V. parahaemolyticus* infections were reported during summer months.

Purpose: The aim of this study was to investigate the fitness of *V. parahaemolyticus* in seawaters at 10°C and 30°C.

Methods: Pathogenic *V. parahaemolyticus* with the *tdh* gene (ATCC 43996) was inoculated into sterile seawater and stored at 10°C and 30°C for 10 days. The fitness of *V. parahaemolyticus* was evaluated by plating surviving cells on thiosulphate-citrate-bile salts-sucrose (TCBS) agar once per day for 10 days at 10°C. At 30°C, *V. parahaemolyticus* was plated every two hours for the first 12 hours, then every four hours for the next 12 hours. On day 2, 3, 5, and 10, *V. parahaemolyticus* was plated once each day. The Baranyi function was used to establish the primary predictive models. Biofilm formation was measured by staining with crystal violet dye and read via Bio-Rad microplate reader.

Results: The established predictive models showed that *V. parahaemolyticus* decreased from 5.25 log CFU/g to 2.67 log CFU/g at 10°C and increased at 30°C from 4.76 log CFU/g to 6.95 log CFU/g after 10 days, with a minimum inactivation rate or maximum growth rate of -0.697 log CFU/day and 0.392 log CFU/day, respectively. The *V. parahaemolyticus* in seawater at 30°C has a significantly higher relative capacity of biofilm formation than 10°C (P<0.05).

Significance: Different fitness of *V. parahaemolyticus* in seawater at different oyster harvesting temperatures was seen. Further preventive control strategies, such as depuration, need to be evaluated to ensure the safety of raw oysters.

P1-73 Migration of Enterohemorrhagic *Escherichia coli* Artificially Internalized into Vegetable Seeds to Different Sections of Sprouts/Seedlings during Germination

DA LIU[1], Ronald R. Walcott[2], Jinru Chen[1] and Yue Cui[1]
[1]*University of Georgia, Department of Food Science and Technology, Griffin, GA,* [2]*University of Georgia, Department of Plant Pathology, Athens, GA*

❖ ***Developing Scientist Competitor***

Introduction: Pathogen-contaminated vegetable seeds have been linked to sprout-related outbreaks of human gastrointestinal infections.

Purpose: In this study, we investigated the migration of enterohemorrhagic *Escherichia coli* (EHEC), artificially internalized into vegetable seeds, to different sprout/seedling tissues during seed germination and seedling emergence.

Methods: Nalidixic acid-resistant EHEC (K4492, H1730, ATCC BAA-2326 and F4546) were artificially infiltrated into alfalfa, fenugreek, lettuce, and tomato seeds using vacuum. Contaminated seeds were germinated on 1% water agar in germination boxes at 25°C for nine days. Ten replicate samples of each seed section, including whole seed, seed coat, cotyledon, stem, and root were collected twice daily. Seed sections were homogenized and aliquots of homogenates were plated, in duplicate, on sorbitol MacConkey agar and tryptic soy agar supplemented with nalidixic acid. The experiment was repeated once and results were analyzed by Fisher's LSD test using the R software.

Results: Cells of all four *E. coli* strains migrated from contaminated seeds to different sprout/seedling tissues. Approximately 67% of the 512 collected seed sections tested positive for *E. coli*. The average population of *E. coli* increased from 0.64 to 1.34 log CFU/seed section during seed germination. On average, the *E. coli* count on whole seeds was significantly lower than the counts from other seed tissues, which were not significantly different from each other (P>0.05). The average populations of the four *E. coli* strains on sprouts and seedlings were not significantly different (P>0.05). *Escherichia coli* counts from alfalfa and fenugreek sprouts (1.81 and 1.34 log CFU/seed section, respectively) were significantly higher (P<0.05) than those from lettuce and tomato seedlings (0.17 and 0.26 log CFU/seed section).

Significance: This study revealed the ability of EHEC to migrate from internal seed tissues to different areas of sprouts/seedlings during germination. It emphasizes the importance of using pathogen-free seeds for commercial vegetable sprout production.

Poster

P1-74 T4 Bacteriophage Insensitive Mutants of *Escherichia coli* Display Altered Antibiotic Resistance and Ability to Ferment Glucose

ZEYAN ZHONG, Anna Colavecchio and Lawrence Goodridge
McGill University, Ste-Anne-de-Bellevue, QC, Canada

Introduction: Bacteriophages (phages) are increasingly used as antimicrobials in foods. The consequences of phage resistance on bacterial fitness in foods remains unknown.

Purpose: The purpose of this study was to evaluate how phage resistance influences the growth properties of *Escherichia coli* in a model system.

Methods: Bacteriophage insensitive mutants (BIMs) of *Escherichia coli* B (ATCC 11303) were produced by incubating suspensions of the bacteria with 10-fold dilutions of an initial 10^{10} PFU/ml stock of phage T4. Six colonies of BIMs (each isolated from different 10-fold phage dilutions) were tested for altered biochemical properties using the VITEK-2 system, susceptibility to antibiotics including extended spectrum beta-lactamases (ESBLs), aminoglycosides (kanamycin) and second generation cephalosporins. Also, growth curves in TSB were conducted for all BIMs.

Results: Compared to wildtype, all BIMs lost the ability to ferment glucose, and were positive for lactate and succinate alkalinisation, suggesting that amino acids in the growth medium were being degraded, resulting in acid stress within the bacterial cytoplasm. A primary mechanism of phage T4 resistance in *E. coli* is to mutate its primary receptors lipopolysaccharide and OmpC, which plays a major role in allowing entry of antibiotics into the bacterial cell. All BIMS were sensitive to ESBLs and cephalosporins but displayed increased MICs for kanamycin (four µg/ml compared to two µg/ml for the wildtype (one BIM had a MIC of eight µg/ml). Growth curves indicated that all BIMs except for one had longer exponential phases than the wild type, and all BIMs had higher ODs (0.85 to 0.9) at 600 nm than the wild type (0.7). Doubling times for the BIMs ranged from 33 to 40 mins in comparison to the wildtype (44 min).

Significance: These results indicate that phage induced mutations may influence fitness of *E. coli* in food, including resistance to antimicrobials that enter the cell through porins.

P1-75 Biofilm-forming Capacity and Resistance to Sanitizers of a Range of *Escherichia coli* O26 Pathotypes from Human Clinical Cases and Cattle in Australia

Salma Lajhar[1], Jeremy Brownlie[1] and **ROBERT BARLOW**[2]
[1]Griffith University, Brisbane, Australia, [2]CSIRO Agriculture & Food, Brisbane, Australia

Introduction: *Escherichia coli* O26 belong to a range of pathotypes including enterohemorrhagic *E. coli* (EHEC), atypical enteropathogenic *E. coli* (aEPEC), nontoxigenic *E. coli* (NTEC) and potential EHEC (pEHEC), some of which are associated with foodborne disease. The ability of *E. coli* to form biofilms can make this organism difficult to eradicate from food production environments.

Purpose: Evaluate the biofilm-forming capacity of *E. coli* O26 isolates, determine whether motility, extracellular matrix (ECM) components and bacterial adhesion to hydrocarbons are factors associated with biofilm formation, and evaluate the sensitivity of isolates to food industry approved sanitizers.

Methods: Forty *E. coli* O26 isolates, comprising 27 EHEC, eight aEPEC, two NTEC and three pEHEC were assessed for their capacity for biofilm formation on polystyrene and at the air liquid interphase under static conditions. Motility, extracellular matrix (ECM) components, and bacterial adhesion to hydrocarbons were also investigated. Finally, the efficacy of three commercially available sanitizers against *E. coli* O26 biofilms was determined.

Results: EHEC isolates were significantly more likely to form biofilms after 48 hours than aEPEC isolates. However, no differences were observed after 72 hours with 28 of 40 (70%) isolates demonstrating biofilm formation. EHEC isolates (63%) were significantly more likely to form a pellicle at the air liquid interphase than aEPEC isolates (0%). Isolates that were motile and expressed ECM components (55%) generated biofilms with significantly greater cell densities than those isolates that were nonmotile and/or did not express ECM components. Overall, no correlation was found between hydrophobicity and biofilm formation. The application of sanitizer resulted in mean reductions in cell density of 0.56 and 0.16 log CFU/ml after 2 and 10 min treatments, respectively.

Significance: EHEC isolates show enhanced capacity to rapidly develop biofilms. The protective advantage that results from biofilm formation may contribute to the frequent association of these isolates with foodborne illness.

P1-76 The Lack of Toll-like Receptor 11 Expression in Mice Does Not Allow for Colonization by Shiga Toxin-producing *Escherichia coli*

LISA HARRISON[1], Prabha Kc[1], Monika Proszkowiec-Weglarz[1], Uma Babu[1], Andrew Do[1], Mohammad Alam[2], Kristina Williams[1] and Kannan Balan[2]
[1]CFSAN, Laurel, MD, [2]U.S. Food and Drug Administration, CFSAN, Laurel, MD

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) continue to be an important public health problem in the United States as they have serious foodborne disease outcomes, including acute renal failure and death. Currently, characterization of STEC is performed using molecular methods and in vitro models due to the lack of suitable animal models.

Purpose: STEC do not cause disease in wild-type (WT) mice, possibly due to several differences between mouse and human intestines. One such difference is the presence of the innate immune receptor Toll-like receptor 11 (TLR11) in mice. In order to determine whether TLR11 prevents STEC colonization and infection in mice, we used TLR11-knockout (KO) mice in STEC challenges.

Methods: WT or TLR11-KO mice were orally gavaged with 10^9 CFU STEC (Ampicillin-resistant O26:H11 strain EC1649) and observed for signs of disease and bacterial shedding. Eight to ten days postchallenge, small intestines, large intestines, ceca, kidneys, spleens, and livers were harvested, homogenized, and plated on ampicillin plates for colony counts.

Results: Both TLR11-KO mice and WT mice showed no signs of distress or disease postchallenge. However, WT mice continued to shed STEC longer than TLR11-KO mice. Organs of TLR11-KO mice contained no detectable STEC, while a few WT mice had low levels of STEC in their large intestines and ceca.

Significance: FDA's food safety mission requires research tools to characterize microbial contaminants in our food supply in order to distinguish between pathogenic and nonpathogenic bacteria. The identification of an animal model for STEC is critical for characterization of disease outcome, elucidating infectious dose of different STEC serotypes, confirming molecular and in vitro methods, and for traceback analyses. This investigation demonstrated that TLR11-KO mice are not a suitable animal model for STEC. It is useful to present negative outcomes so that animal challenges are not unnecessarily repeated.

P1-77 Effect of Adaptation to Sublethal Concentrations of Acetic Acid and pH on Serovar- and Strain-dependent Acid Resistance of *Salmonella* spp.

ALKMINI GAVRIIL[1], Adamantia Papaioannou[1], Nefeli Lysimachou[2] and Panagiotis Skandamis[1]
[1]*Agricultural University of Athens, Athens, Greece,* [2]*Technological Educational Institute of Athens, Athens, Greece*

❖ ***Developing Scientist Competitor***

Introduction: Stress hardening of microorganisms as a result of mild treatments during food processing can lead to enhanced survival against lethal stresses. This may eventually reduce the efficiency of hurdle technology and compromise food safety.

Purpose: To investigate the acid resistance of different serovars and strains of *Salmonella* spp. in mayonnaise after adaptation to sublethal concentrations of acetic acid and pH values.

Methods: Two strains of *Salmonella* Enteritidis and three strains of *Salmonella* Typhimurium were tested. Following growth in tryptic soy broth without glucose (TSB Glu(-)), cells were transferred to TSB Glu(-) supplemented with 15, 35, and 45 mM total acetic acid (AA). The pH of the medium was adjusted to values 5.0, 5.5, and 6.0, respectively, resulting in different concentrations of undissociated AA. After incubation for 90 minutes at 37°C, the cultures were inoculated to commercial packages of mayonnaise and the samples were stored at 5°C. Nonadapted (NA) inocula were grown in nonacidified TSB-Glu(-).

Results: The time needed for the reduction below the enumeration limit (<two log) was affected by the serovar tested. Both *Salmonella* Enteritidis strains were more acid sensitive compared to *Salmonella* Typhimurium strains, since they remained countable for 24 to 46 hours depending on the strain and the treatment tested, while *Salmonella* Typhimurium strains were enumerated for up to five to seven days on a general purpose medium supplemented with sodium pyruvate**,** depending again on the strain and the treatment tested. For some of the strains used, differences in the acid resistance were observed between NA inocula and some adaptation treatments. For example, adaptation at 45mM/pH6.0 resulted in higher population levels (P<0.05) compared to NA cells for one strain of *Salmonella* Enteritidis.

Significance: This study may provide new insights for the role of undissociated acetic acid and its impact on *Salmonella* stress response and survival in acetic acid based products.

P1-78 Evaluation of Selective and Nonselective Plating Media for Recovery of *Salmonella* Enteritidis PT 30, *Salmonella* Seftenberg 775W, and *Salmonella* Typhimurium DT 104 Colonies from Heat-treated Almonds

BUKOLA ONARINDE, Pauline Lovatt, Yunus Khatri and Gerrit Meerdink
University of Lincoln, Lincoln, United Kingdom

Introduction: Validation studies aimed at determining the effect of a thermal process on survivability of a pathogen requires a reliable enumeration method to successfully determine the survivability during food processing. Direct plating method have been utilised in many research for determining the initial and final level of microorganisms in order to calculate the microbial log reduction achieved during a process validation study. Also research have shown that *Salmonella* species could become heat injured during thermal processing which makes it difficult to recover their cells.

Purpose: This study was designed to assess the efficiency of a non-selective agar; Tryptone Soya Agar (TSA) and five selective media; Bismuth Sulfite Agar (BSA), Chromogenic Agar (CHR), Hektoen Enteric Agar (HEA), MacConkey Agar (MAC) and Xylose Lysine Deoxycholate Agar (XLD) for the detection, enumeration and recovery of *Salmonella* before and after heat treatment.

Methods: Artificially inoculated almonds were roasted in rapeseed oil heated to approximately 120°C for 95 seconds. The population of *Salmonella* before and after heat treatment was then enumerated by direct plating on TSA, BSA, CHR, HEA, MAC and XLD.

Results: The mean level of *Salmonella* (n=4) before heat treatment ranged from 8.69 to 9.68 log cfu/g of almonds for the three serovars. After heat treatment (n=4) log cfu/g level of *Salmonella* Enteritidis PT 30 (SEPT 30), *Salmonella* Seftenberg 775W (775W) and *Salmonella* Typhimurium DT 104 (DT 104) recovered ranged from 5.01±0.01(HEA) to 5.55±0.26(TSA), 4.01±0.20(XLD) to 5.16±0.30(TSA) and 4.82±0.15(CHR) to 5.84±0.05(TSA), respectively. The media that were optimal for the recovery of SEPT 30 and 775W included TSA, BSA and MAC, which recovered more cells (P≤ 0.01) than CHR, HEA and XLD. For DT 104, TSA, HEA and MAC recovered more cells.

Significance: The result obtained in this study suggests the use of TSA in combination with BSA and/or MAC agar for maximum recovery of *Salmonella* cells after oil roasting process.

P1-79 Metabolomic Analysis of Electron Beam Inactivated *Salmonella* Typhimurium

SOHINI BHATIA[1] and Suresh D. Pillai[2]
[1]*Texas A&M University, College Station, TX,* [2]*National Center for Electron Beam Research, College Station, TX*

❖ ***Developing Scientist Competitor***

Introduction: Outbreaks of *Salmonella* Typhimurium continue to be a leading cause of foodborne illness worldwide. Although food safety interventions, such as ionizing radiation, are implemented and are highly effective in inactivating pathogens such as *Salmonella*, these pathogens can remain metabolically active.

Purpose: Understanding how the metabolomic activity of irradiated cells changes over time can help the food industry better exploit these metabolically active yet nonculturable (MAyNC) cells for their immune modulating capabilities.

Methods: A 10^9 CFU/ml titer of *Salmonella* Typhimurium (ATCC 13311) was suspended in phosphate buffered saline and irradiated at a lethal target dose of two kGy. Culture methods were used to confirm the nonculturable state of the irradiated cells. Untargeted metabolomic analysis was performed on unirradiated cells, freshly irradiated cells, and irradiated cells that were incubated at room temperature for 24 hours.

Results: Three hundred forty-nine metabolites were identified using untargeted metabolomic analysis. Eighty-eight of these were expressed at statistically different concentrations (P<0.01) between the treatment groups. Pathways that were impacted (P<0.01) include Glyoxylate/Dicarboxylate metabolism, Arginine/Proline metabolism, Glycine/Serine/Threonine metabolism, beta-Alanine metabolism, Alanine/Aspartate/Glutamate metabolism, and Inositol phosphate metabolism.

Significance: These results show that the metabolomic profile of irradiated *Salmonella* Typhimurium greatly differs between unirradiated cells, freshly irradiated, and irradiated cells that were incubated for 24 hours. The pathways elicited suggest that not only does ionizing radiation have an effect on the metabolites being produced within the cell, but this stress continues to cause changes even after 24 hours at room temperature. The immune modulating properties of these inactivated pathogens in food is worthy of investigation.

P1-80 Survival and Heat Resistance of *Salmonella* during Simulated Commercial Manufacturing of Tortillas

MINTO MICHAEL[1], Daniel Vega[1], Keyla Lopez[1], Jennifer Acuff[1], Lakshmikantha Channaiah[2], George Milliken[1], Harshavardhan Thippareddi[3] and Randall Phebus[1]

[1]Kansas State University, Manhattan, KS, [2]AIB International, Manhattan, KS, [3]University of Georgia, Athens, GA

Introduction: Flour is generally not pasteurized and can become contaminated with pathogens such as *Salmonella*. These pathogens can survive in flour for extended periods; therefore, it is important to utilize a validated cooking step to eradicate pathogens during manufacture of ready-to-eat tortillas.

Purpose: This study validated a simulated commercial tortilla cooking process to control a seven-serotype *Salmonella* (Hartford, Newport, Senftenberg, Tennessee, Typhimurium, and two isolates from pet food) cocktail, and determined *D*- and z-values of the cocktail in tortilla dough.

Methods: Wheat flour was mist inoculated with the *Salmonella* cocktail, dried to the pre-inoculation weight to achieve ~7.5 log CFU/g, and used for preparing tortilla dough. For the cooking validation, dough was pressed in a standard tortilla press (top and bottom plates at ~93 and 43°C, respectively), heated on a pan (preset at 240°C) for 30, 45, or 60 s on each side, followed by 10 min of ambient cooling. *D*-values of the *Salmonella* cocktail were determined at 55, 58, and 61°C using thermal-death-time disks. Both studies utilized a randomized complete block (three replications) design and analyses were conducted at $P \leq 0.05$.

Results: After cooking for 30, 45, or 60 s on each side, the *Salmonella* population decreased by >7.5 log CFU/g in flour tortillas; however, *Salmonella* was detected by enrichment plating at the end of each cooking time. *D*-values of the *Salmonella* cocktail in tortilla dough at 55, 58, and 61°C were 22.19, 13.48, and 4.59 min, respectively; the *z*-value was 8.85°C.

Significance: This study validated that >7.5 log reduction of *Salmonella* in flour tortillas is achieved after cooking for ≥30 s on each side at a 240°C pan temperature. The calculated *D*- and *z*-values in this study can help the flatbread industry design appropriate parameters to control *Salmonella* contamination in ready-to-eat tortillas.

P1-81 Comparison of Heat Resistance of *Salmonella* during Simulated Commercial Baking of Hard and Soft Cookies

MINTO MICHAEL[1], Jennifer Acuff[1], Keyla Lopez[1], Lakshmikantha Channaiah[2], George Milliken[1], Harshavardhan Thippareddi[3] and Randall Phebus[1]

[1]Kansas State University, Manhattan, KS, [2]AIB International, Manhattan, KS, [3]University of Georgia, Athens, GA

Introduction: Low moisture bakery ingredients, such as flour, sugar, egg solids and milk powders, can become contaminated with *Salmonella*; therefore, validated baking processes should be utilized to ensure finished product safety.

Purpose: This study validated simulated commercial baking processes for hard and soft cookies to control *Salmonella*, and determined *D*- and *z*-values of a seven-serovar *Salmonella* (Hartford, Newport, Senftenberg, Tennessee, Typhimurium, and two pet food isolates from pet food) cocktail in cookie batters.

Methods: Cookie batters were prepared using flour mist-inoculated with the *Salmonella* cocktail (ca. seven log CFU/g). Hard and soft cookies were baked at 185°C for 16 min and 165.6°C for 22 min, respectively, followed by 30 min of ambient cooling. *D*-values of the cocktail in cookie batters were determined using thermal-death-time disks. Studies were designed as randomized complete blocks with three replications as blocks (a=0.05).

Results: *Salmonella* populations decreased by >five log CFU/g in hard and soft cookies at 11.5 and 20.5 min of baking, respectively. *Salmonella* was completely eliminated in hard cookies at the end of baking (as determined by enrichment), whereas in soft cookies, 0.64 log CFU *Salmonella*/g was present at the end of baking and cooling. *Salmonella D*-values in hard cookie batter at 60, 65, and 70°C were 59.57, 28.08, and 11.85 min, respectively; while in soft cookie batter they were 62.29, 28.59, and 14.36 min, respectively. The *Salmonella z*-values in hard and soft cookie batters were 14.53 and 15.77°C, respectively.

Significance: This study validated the baking processes for hard and soft cookies; however, additional research should be conducted if inclusions such as dry fruits, nuts or chocolates are added in to the recipe, as these ingredients might impact the *Salmonella* lethality. The *D*- and *z*-values determined in cookie batters can be used by the bakery industry to evaluate the expected lethality of their baking schedules.

P1-82 Validation of a Frying Process to Control *Salmonella* in Donuts

MINTO MICHAEL[1], Jennifer Acuff[1], Keyla Lopez[1], Daniel Vega[1], George Milliken[1], Harshavardhan Thippareddi[2], Randall Phebus[1] and Lakshmikantha Channaiah[3]

[1]Kansas State University, Manhattan, KS, [2]University of Georgia, Athens, GA, [3]AIB International, Manhattan, KS

Introduction: Nonpasteurized, low moisture food ingredients, such as flour, sugar and milk powders, can be contaminated with pathogens such as *Salmonella*; therefore, FSMA mandates that all preventive control processing steps for ready-to-eat human foods be validated to control these foodborne pathogens.

Purpose: This study validated a simulated commercial donut frying process against a seven-serovar *Salmonella* (Hartford, Newport, Senftenberg, Tennessee, Typhimurium, and two pet food isolates) cocktail, and determined the thermal inactivation parameters (*D*- and *z*-values) of the *Salmonella* cocktail in donut dough.

Methods: Bread and pastry flour were mixed, inoculated with *Salmonella* cocktail, dried to preinoculation weight to achieve ~7.5 log CFU *Salmonella*/g population, and used to prepare donut dough. The donut frying process was validated using a 190.6°C oil temperature for one minute on each side followed by 30 minutes of ambient air-cooling. Population reductions were determined based on initial raw dough pathogen levels. *Salmonella* cocktail *D*-values in donut dough were determined using thermal-death-time disks and hot-water bath. Both studies utilized randomized complete block designs with three replications as blocks ($P \leq 0.05$).

Results: The *Salmonella* population decreased by approximately one log CFU/g in donuts after one minute of frying; however, *Salmonella* was completely eradicated by the end of two minutes of frying. The *D*-values of the *Salmonella* cocktail in donut dough at 55, 58, and 61°C were 8.55, 2.87, and 2.13 min, respectively. The *z*-value was 10.03°C.

Significance: This studied simulated a commercial donut frying process and achieved >7.5 log CFU *Salmonella*/g reduction in finished donuts when fried for one minute on each side at ~190.6°C oil temperature. As natural *Salmonella* contamination of ingredients, such as flour, is likely considerably <7 log CFU/g, frying donuts utilizing these validated parameters will provide a high level of assurance on product safety relative to *Salmonella* or similar vegetative pathogens.

P1-83 Reduction of *Salmonella* spp. and Shiga Toxin-producing *Escherichia coli* on Alfalfa Seeds and Sprouts Using an Ozone Generating System

ZAHRA MOHAMMAD, Ahmad Kalbasi and Alejandro Castillo
Texas A&M University, College Station, TX

❖ ***Developing Scientist Competitor***

Introduction: Several outbreaks have been associated with consumption of alfalfa sprouts contaminated with *Escherichia coli* and *Salmonella.* Sprouts have become public health concern. Since chemical treatments to inactivate pathogens on alfalfa seeds and sprouts have shown little effect, the ozone application was investigated as an intervention.

Purpose: The purpose of this study was to evaluate the application of ozone to reduce Shiga-toxigenic *E. coli* (STEC) and *Salmonella* on the surface of alfalfa seeds and on sprouts.

Methods: Seeds inoculated with a cocktail of three strains of *Salmonella* and three strains of STEC and sprouts obtained from the same inoculated seeds were separately subjected to aqueous ozone treatment containing five mg/liter ozone for 10, 15, 20 minutes. The samples were immersed into ozonated water with continuous pressurized (10 psi) oxygen feeding.

Results: The mean log reductions achieved after treatment were (1.6±0.2, 1.7±0.3, and 2.1±0.5) and (1.5±0.4, 1.6±0.4, and 2.1±0.5) for *Salmonella* and STEC, respectively. For sprouts obtained from the inoculated seed, the log reductions after treatment for 10, 15, and 20 min were (0.7±0.2, 1.1±0.4 and 3.6±0.1) and (0.7±0.1, 1.2±0.3 and 1.8±0.1) for *Salmonella* and STEC, respectively. There were significant differences between log reductions after the different times of treatment in both seeds and sprouts. There were no significant difference (P>0.05) between *Salmonella* and STEC in the log reductions achieved on seeds. However, there were significant differences between *Salmonella* and STEC in the log reductions achieved on sprouts. In addition, visual examination showed no negative effects on ozone treatment on seeds or sprouts.

Significance: Ozone may be a feasible intervention to eliminate STEC and *Salmonella* from the surface alfalfa seeds and sprouts if used with appropriate concentration and proper time of exposure.

P1-84 Concentration-dependent Neutralization of Antimicrobials Used in Poultry Processing Allowing Survival of *Campylobacter* spp.

ZAHRA MOHAMMAD, Matt Taylor and Christine Alvarado
Texas A&M University, College Station, TX

❖ ***Developing Scientist Competitor***

Introduction: Antimicrobials applied to poultry carcasses during harvest and fabrication may result in residues of antimicrobials being carried over into carcass rinsing fluid, decreasing the likelihood of pathogen detection during routine testing. Antimicrobial neutralizers, when used, may assist the detection of pathogens, like *Campylobacter* spp., on fresh poultry.

Purpose: The purpose of this study was to test the abilities of antimicrobial-specific neutralizers applied, at systematically increasing concentrations, to cetyl pyridinium chloride (CPC)- or peracetic acid (PAA)-exposed *Campylobacter jejuni* to determine minimum neutralizer concentrations necessary to allow pathogen survival.

Methods: *Campylobacter jejuni* were inoculated to a target of approximately five log CFU/ml in double strength Mueller Hinton Broth (2X MHB) and, then, immediately exposed to CPC at 0.1 to 0.8% (w/v; final concentration), or PAA at 0.0125 to 0.2% (0.2%=2,000 ppm; final concentration). For CPC, a combination of lecithin and Tween 20 was added to buffered peptone water (BPW) at 0.125 to 2.0X their standard concentrations in Dey-Engley (D/E) Neutralizing Buffer. For PAA, disodium phosphate (Na_2HPO_4) and potassium monophosphate (KPO_4) were added at 0.25 to 3.0X their standard concentrations in BPW. Sodium thiosulfate was also added at 0.15 to 1.8% (w/v) to neutralize peroxy-radicals. Test tubes were incubated for 24 h at 35°C and, then, inspected for turbidity as indicative of pathogen growth or inhibition.

Results: At 0.8% CPC, 1X CPC-neutralizer additions were required to observe growth for *Campylobacter* isolates across replicates. Conversely, for PAA the addition of neutralizers to the 2X application was required to allow *Campylobacter*survival at 2,000 ppm applied PAA.

Significance: Increasing concentrations of sanitizers consistently required higher concentrations of neutralizers to counteract sanitizer antimicrobial activity, allowing *Campylobacter* growth and detection. Antimicrobial neutralizers, when added to poultry rinsing fluids, may improve the detection of viable *Campylobacter* from fresh poultry.

P1-85 Comparison of Survival and Heat Resistance of *Escherichia coli* O121 and *Salmonella* in Muffins

Minto Michael[1], Jennifer Acuff[1], **DANIEL VEGA**[1], Keyla Lopez[1], George Milliken[1], Harshavardhan Thippareddi[2] and Randall Phebus[1]
[1]Kansas State University, Manhattan, KS, [2]University of Georgia, Athens, GA

Introduction: Shiga-toxigenic *Escherichia coli* and *Salmonella* can survive in flour for the extended periods; thus, thermal lethality processes in bakery operations are important to ensure the safety of products. Published studies comparing the thermal tolerance of STEC to *Salmonella* in bakery products are lacking.

Purpose: This study validated a simulated commercial muffin baking process against *E. coli* O121 (flour outbreak isolate), and determined *D*- and *z*-values of *E. coli* O121 (four-isolate cocktail) and *Salmonella* (three-serovar cocktail of Newport, Typhimurium, and Senftenberg) in muffin batter.

Methods: Batter were prepared using spray inoculated flour containing ~7 log CFU *E. coli* O121 or *Salmonella*/g. For the baking validation, batter was baked at 190.6°C oven temperature for 21 min followed by 30 min of ambient cooling. *D*-values of *E. coli* O121 and *Salmonella* cocktails in batter were determined using thermal-death-time disks. Randomized complete block designs were used for each study, with three replications as blocks (a=0.05).

Results: *Escherichia coli* O121 populations decreased by >7 log CFU/g in muffins at 17 min, and were completely eliminated at 21 min of baking. *D*-values of *E. coli* O121 and *Salmonella* cocktails in muffin batter at 60, 65, and 70°C were 42.03 and 38.38, 7.49 and 7.17, and 0.42 and 0.47 min, respectively. The *z*-values of *E. coli* O121 and *Salmonella* were 5.01 and 5.22°C, respectively.

Significance: Survival of *E. coli* O121 in muffins during oven baking was slightly lower than observed for *Salmonella*, as determined in a previous study by these authors. The validated baking process against *Salmonella* would effectively control similar levels of *E. coli* O121. Both organism cocktails demonstrated similar *D*- and *z*-values in batter and can be used by the bakery industry to establish safe baking protocols; however, specific validation studies may be appropriate if the baking parameters or muffin recipe are modified.

P1-86 Efficacy of the InnovaPrep Concentrating Pipette for Concentrating *Salmonella* spp., *Listeria* spp., and *Escherichia coli* in Ground Beef and Leafy Greens for Rapid Detection

GIANNA PRATA, Patrick Marek, Tobyn Branck and Christina Crivello
Natick Soldier Research Development & Engineering Center, Natick, MA

Introduction: Rapid, single day detection of foodborne pathogens in leafy greens and raw ground beef is needed by the Armed Services and food industry. The InnovaPrep Concentrating Pipette (CP) is of interest due to its ability to concentrate bacteria from a food homogenate, replacing older microbiological methods including centrifugation and enrichment as a front-end to microbial detection.

Purpose: The objective of this study was to determine the ability of the InnovaPrep CP technology to extract and concentrate bacteria from a food matrix for detection in less than eight hours.

Methods: Six experimental runs of eight replicates containing an inoculum of one to 10 CFU/25 g food were sampled using the InnovaPrep CP at time points zero, four to six, and 24 h post enrichment. All samples were processed for DNA extraction with an iQ-Check Biorad kit for *Salmonella* spp., *Listeria* spp., or *Escherichia coli* O157:H7. Standard sampling after enrichment in selective broths, at each time point, was conducted as a control. PCR was performed and positive samples were determined by endpoint analysis using the negative control RFU plus the tolerance determined by the IQ5 software.

Results: There were significant differences ($P \leq 0.05$) in ground beef inoculated with *E.coli* at zero and four hours and lettuce inoculated with *L. monocytogenes* at zero hours using the CP versus traditional sampling methods.

Significance: Our data showed that the InnovaPrep CP system can detect one to 10 CFU/25g food in a zero to six hour time frame, but the results are inconsistent and food specific. These results are relevant as this technology is of interest to the food industry as a means for rapid pathogen detection.

P1-87 Improved Recovery of *Salmonella* spp. and *Cronobacter* spp. in Dry Milk Powders Enriched in Brilliant Green Water Compared to Buffered Peptone Water

Philip Feldsine, Markus Jucker, Mandeep Kaur, Amy Immermann and **ANDREW LIENAU**
BioControl Systems, Bellevue, WA

Introduction: *Salmonella* and *Cronobacter* are two pathogens of concern in infant nutritionals, with one major ingredient being milk powders. The ISO methods for *Salmonella* and *Cronobacter*, as well as the FDA method for *Cronobacter*, enrich milk powders in Buffered Peptone Water (BPW). Previous studies have shown improved recovery of *Salmonella* in milk powders when enriched in Brilliant Green Water (BGW), prompting the FDA to enrich milk powders in BGW. It is important to determine for milk powders if BGW also improves recovery of the *Salmonella* and *Cronobacter* when compared to the standard enrichments in BPW.

Purpose: To evaluate the recovery of *Salmonella* and *Cronobacter* in milk powders enriched in BGW compared to BPW.

Methods: Each lyophilized organism was inoculated into bulk dry milk powder, stabilized at room temperature for minimum of two weeks, then diluted for partial recovery. To create composite sized samples (either 100 g or 375 g), 25 g of base inoculated milk powder was mixed with uninoculated dry milk to achieve the final size. Samples were enriched 1:10 in media for 20 to 24 h.

Results: For *Salmonella*, 24 milk powder samples were enriched in BPW and BGW. The recovery in BPW was 13, while the recovery in BGW was 17 of 24 samples. For *Cronobacter*, 38 samples were enriched in BPW and BGW. The recovery in BPW was 20 while the recovery in BGW was 38 of 38 samples. While recovery in BGW was complete for *Cronobacter*, much lower partial recovery occurred when enriched in BPW.

Significance: The improved recovery of *Salmonella* and more dramatically for *Cronobacter* in BGW is important to achieve the maximum recovery and detection in milk powders. Given the use of milk powders as a key ingredient in the manufacture of infant nutritionals, this new enrichment condition should be considered superior for microbiological analysis of milk powders. Further, BGW is an economic, simple and well-established medium, increasing its acceptance.

P1-88 Effect of Ultraviolet C Light on the Reduction of *Aspergillus* and *Penicillium* Species on Moist and Dry Surfaces

HASSAN GOURAMA
Penn State University, Reading, PA

Introduction: Due to its mutagenic properties, ultraviolet (UV) light has been used for many years to inactivate microorganisms on surfaces in many industries. Short wavelengths (250 to 260 nm) of ultraviolet light (UVC) are considered the most germicidal. Prior research explored the inactivating properties of UVC on a number of foodborne fungi. However, there are still many fungal species that have not been fully investigated.

Purpose: The aim of this investigation was to evaluate the effect of UVC light on *Aspergillus* and *Penicillium* species on moist and dry surfaces.

Methods: Ten *Aspergillus* and ten *Penicillium* species were used in this study. For the moist surface method, 0.1 ml of mold spore suspensions (~ 10^2 to 10^3 spores/plate) were spread on MEA plates and exposed to UVC treatment (254 nm) for 0, 5, 10, 15, 30, 60, 120, and 150 seconds. For the dry surface method, mold spores (~ 10^5 spores) were dried onto a membrane filter, and then treated with UVC (254 nm) for 0, 30, 60, 120, 180, and 300 seconds. The membranes were placed in 0.1% sterile peptone water, mixed in a stomacher, diluted, and plated onto MEA plates. All plates were incubated at 25°C for three days. Colonies were counted. All the experiments were done in triplicate.

Results: On moist surfaces, after 30 seconds exposure, 70 to 99% reductions were observed for most of the species tested. On the dried filter membranes, a reduction of viable spores of up to 3.6 log was observed after 180 s exposure. Mold species with dark pigmented spores, such as *Aspergillus carbonarius,* were more resistant to UVC. *Penicillium* species were significantly ($P<0.001$) more sensitive to UVC, with approximately three-log reduction in comparison with one to two-log reduction for the *Aspergillus* species.

Significance: UVC light treatment is an effective process for inactivating mold spores on moist and dry surfaces. Longer exposure periods should be used for dry surfaces.

P1-89 Microbiological Evaluation in Infant Formulas Powdered and Reconstituted at Home

ADRIANE NARUMI ONODERA ANDRADE[1], Eliezer Flavio Do Nascimento Andrade[1], Rosana Francisco Siqueira Dos Santos[1], Agueda Cleofe Marques Zaratin[1] and Ana Valeria Ulhano Braga[2]

[1]Metrocamp College Devry Group, Campinas, Brazil, [2]University of Campinas (UNICAMP), Campinas, Brazil

Introduction: Infant formula is a milk based food used as substitute for breast milk, and is generally the only source of nutrients for children under six months of age. Microbiological contamination of infant formulas can cause high-risk diseases in these children due to the immaturity of their immune system.

Purpose: Evaluate the microbiological quality of powdered and reconstituted infant formulas prepared at home.

Methods: Forty-two samples (22 of powdered formula and 22 of the same batch reconstituted prepared by the mothers) were analyzed for the presence of *Enterobacteriaceae*, *Escherichia coli*, *Bacillus cereus*, and *Cronobacter*spp.

Results: Two samples of powdered formulas (9.1%) had *Enterobacteriaceae* counts, ranging from $1.7x10^2$ to $7.0x10^2$ CFU/g. One sample was contaminated with *E. coli* (56 CFU/g), the other with *B. cereus* (10 CFU/ml). *Enterobacteriaceae* were isolated from 16 samples of reconstituted formulas (72.7%) with results varying from $4.4x10^2$ to $1.0x10^5$ CFU/ml, and from that samples, seven had *E. coli* with results ranging from $6.0x10^2$ to $5.9x10^4$ CFU/ml. *Bacillus cereus* was present in five samples (22.7%); three had counts of 50, 80 and 100 CFU/ml. *Cronobacter* spp. was not isolated from the samples; however, *Klebsiella pneumoniae* (one powdered sample and two reconstituted) and *Pseudomonas aeruginosa*(two powdered sample and one reconstituted) were isolated during the analysis.

Significance: The presence of these microorganisms can cause gastro intestinal diseases in infants, which can progress to severe infections such as meningitis and sepsis as they have immature immune systems. The contamination of the samples was greater in the reconstituted formulas, showing that there is a need for a greater hygienic-sanitary control during the preparation. It is important that mothers who prepare the food are oriented to the hygiene of the utensils, ideal temperature of reconstitution and temperature in which the formula is exposed, avoiding the transmission of diseases to their children.

P1-90 Microbial Safety of Human Milk Purchased from Online Markets

DONG JOO SEO[1], Hyunkyung Park[2], Suntak Jeong[2], Hanseam Shin[3] and Changsun Choi[4]

[1]Chung-Ang University, Food & Nutrition, Anseong, Kyounggi, South Korea, [2]Chung-Ang University, Anseong, South Korea, [3]Chung-Ang University, Ansung, South Korea, [4]Chung-Ang University, Ansung, Kyounggi, South Korea

Introduction: As the importance of breastfeeding is emphasized to educate mothers, private or online sale of human milk has been increased because of various reasons including environmental and biological factors such as allergenic reaction to the milk powder, problem of breastfeeding, and health problem of infants or mother. However, the lacking of microbiological standard in human milk traded in online market can be the great health risk in infants.

Purpose: The aim of this study was to evaluate microbiological safety in human milk sold from online market.

Methods: Eighty five human milk samples were purchased from online market. Sixty two human milks and 23 human milks were obtained by direct or online purchase. Total bacteria, coliforms, *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., and *Streptococcus* spp. were monitored in each human milk sample. The contamination level of each microorganism was analyzed according to purchased route.

Results: Total aerobic bacteria and coliforms was ranged from zero to 5.6 log CFU/ml and from zero to 4.0 log CFU/ml in 85 human milks, respectively. Two human milks were contaminated with 2.8 and 3.6 log CFU *E. coli*/ml. Detection range of *Staphylococcus* spp. was zero to 4.7 log CFU/ml in 64 human milks. Neither *Salmonella* spp. or *Streptococcus* spp. were detected in human milks. While the detection rate of each microorganism between direct and online purchase was not significantly different, the contamination level of human milks purchased from online was higher than that of human milks obtained by direct purchase.

Significance: This study confirmed that the contamination of *E. coli* and *S. aureus* was the major microbial risk of human milk traded in online market.

P1-91 Histamine Production by *Photobacterium* spp.

KRISTIN BJORNSDOTTIR-BUTLER[1], Paul V. Dunlap[2] and Ronald A. Benner, Jr[1]

[1]FDA Gulf Coast Seafood Laboratory, Dauphin Island, AL, [2]University of Michigan, Ann Arbor, MI

Introduction: Scombrotoxin (histamine) fish poisoning (SFP) is the most frequent cause of fish poisoning illness in the United States. *Photobacterium* spp. have been implicated in SFP outbreaks. However, possible misidentification of isolates and the discovery of new *Photobacterium* spp. have led to confusion as to which species within the genus are capable of producing histamine.

Purpose: The purpose of this study was to identify *Photobacterium* spp. capable of producing histamine at levels of human health concern (≥200 to 500 ppm).

Methods: Histamine production of 108 *Photobacterium* strains from 24 species was determined after incubation in 10 ml of TSB containing 1% histidine, at 20°C for 48 h with 200 rpm shaking. Histamine was determined using the modified AOAC fluorometric method (977.13). The presence of the histidine decarboxylase (*hdc*) gene was determined by real-time PCR. Selected *Photobacterium* spp. were shotgun sequenced by Ion Torrent Instrumentation to confirm the presence of the *hdc* gene.

Results: Four *Photobacterium* spp. had strains that were able to produce histamine concentrations of human health concern (≥200 to 500 ppm); *Photobacterium angustum* (1 of 12), *Photobacterium aquimaris* (2 of 3), *Photobacterium kishitanii* (20 of 20), and *Photobacterium phosphoreum* (3 of 19). All histamine-producing *Photobacterium* spp., except *P. phosphoreum,* were also positive by real-time PCR for the presence of the *hdc* gene. Shotgun sequencing did not identify the *hdc* gene in the three histamine-producing *P. phosphoreum* strains.

Significance: Results indicated that there may be variation in the *hdc* gene or other genes responsible for histamine production by *P. phosphoreum* strains. In addition, recognizing the histamine-producing species is important to characterizing conditions that can lead to toxic levels of histamine production in fish.

P1-92 Evaluation of Composite Sterility Testing Procedures for Ready-to-Eat Pudding Products

CHRISTOPHER SHOWALTER, Balasubrahmanyam Kottapalli, Kari Sweeney and Deann Akins-Lewenthal
Conagra Brands, Omaha, NE

Introduction: Sterility testing is a common practice in the manufacturing of aseptically processed products to verify that upstream preventive controls are effective. The current testing protocol for ready-to-eat (RTE) pudding products at Conagra Brands requires a minimum of 275 pudding samples (per lot) to be tested using a bioluminescence-based screening method. However, given manufacturing practices, product formulations and lot size the required testing per lot may exceed 500 samples. Compositing of pudding samples provide manufacturing facilities a feasible way to conduct sterility testing and maintain a high level of statistical confidence without increasing testing costs. Currently, there is limited scientific basis to support composite testing.

Purpose: The purpose of this study was to develop a scientifically valid composite testing protocol per FSMA to perform sterility testing of RTE pudding products.

Methods: Two pudding formulations (vanilla and chocolate) were used for the study. Pudding samples were individually inoculated with a three strain gram-negative cocktail and a four strain gram-positive cocktail at target levels of 1.0 to 2.0 log cfu/pudding cup and incubated three days at 35°C. Samples were analyzed using Celsis® RapidScreen® per manufacturer's instructions and confirmed with FDA-approved methods. Samples were then composited in levels of one to five samples (one part inoculated positive sample combined with zero to four parts uninoculated negative samples). Alpha-amylase as a process aid (should the enzyme be added before or after incubation) was also evaluated as part of the study. The study constituted three replicates.

Results: This study indicated that compositing of pudding samples (levels one to five) regardless of the addition of alpha-amylase before or after incubation resulted in 100% detection of the all the organisms evaluated in this study. However, samples where amylase was added post-incubation resulted in bioluminescence values significantly ($P<0.05$) lower compared to their counter parts.

Significance: These findings provide scientific basis for composite testing protocols utilized by Conagra Brands for sterility testing of RTE pudding products.

P1-93 Persistence of Fecal Indicator Bacteria and *Bacteroidales* Universal Marker on Two Different Texture Surfaces

GILBERTO ORDAZ, Angel Merino, Santos Garcia and Norma Heredia
Universidad Autonoma de Nuevo Leon, San Nicolas, Mexico

❖ ***Developing Scientist Competitor***

Introduction: To reduce the risk of produce contamination, fecal indicator bacteria (FIB) are usually quantified, but the source of contamination is not clarified with this method. Members of *Bacteroidales* have been recognized as a promising option for microbial source tracking (MST). However, persistence of *Bacteroidales* has been poorly studied in foods and food surfaces.

Purpose: To determine the persistence of *Bacteroidales* and traditional FIB on two different texture surfaces under two storage conditions.

Methods: Pools of sterile feces were inoculated with *Enterococcus faecalis ATCC* 19433 (8.84 log CFU/ml), *Escherichia coli* ATCC 25922 (10.30 log CFU/ml) and *Bacteroides thetaiotamicron* (5.34 log CFU/ml). Aliquots were placed on 47 mm Millipore filters and 47 mm flat cork circles. Samples were maintained in a bioclimatic chamber at 10°C/95% RH (T1) or 25°C/65% RH (T2) for 25 days. Each five days samples were washed and the rinsate plated onto selective agars (*Streptococcus* KF and Rapid'*E. coli* 2) and the presence of *Bacteroidales* universal AllBac marker was analyzed by qPCR. Assays were done three times and analyzed by SPSS 21.

Results: Without following any pattern, AllBac marker was present in all rinsates until day 25. The detection levels ranged from 1.16 to 4.71 log CFU/ml, independently of the surface texture, feces or storage conditions. Viability of FIB, were reduced during the treatments, *E. coli* by 1.48 to 4.26 log CFU/ml and 6.06 to 6.30 log CFU/ml at T1 and T2, respectively, and population of *E. faecalis* was reduced 0.56 to1.39 log CFU/ml at T1 and 2.3 to 4.0 in T2. At T1 both FIB persisted until the end but at T2 *E. coli* was not recovered on days 10 to 15. FIB persistence were dependent to humidity and species of bacteria ($P<0.05$).

Significance: This study shows that AllBac marker could persist onto surfaces longer than some FIB and could be a good alternative for MST.

P1-94 Independent Evaluation of Prepared Gamma Irradiated Dehydrated Culture Media to Traditional Bulk Dehydrated Culture Media from Various Manufacturers

PATRICK BIRD[1], Tony Gonzalez[2], Joe Benzinger[1], Erin Crowley[1], James Agin[1] and David Goins[1]
[1]Q Laboratories, Inc., Cincinnati, OH, [2]Millennium Life Sciences, Anaheim, CA

Introduction: Microorganisms require nutrients, energy source and certain environmental conditions in order to grow. Due to the diversity of microorganisms, there are numerous types of media required for growth. Prior to use, media often requires a sterilization step, e.g. autoclave sterilization; however, autoclave sterilization can lead to the breakdown of certain carbohydrates. A new technique employing gamma irradiation has been adopted as a viable alternative that uses water soluble polyvinyl alcohol (PVA) film pouches (inert in solution) fabricated to hold the dehydrated media eliminating the need for autoclave sterilization.

Purpose: Evaluation of growth parameters (appearance, growth promotion, sterility and pH) in order to compare commercially available dehydrated media and one ready-to-use PVA media.

Methods: Nine different types of media, including bulk dehydrated culture media from two manufacturers and the alternative PVA media pouches were evaluated. Three separate batches of each media were prepared and evaluated in triplicate. For growth promotion, replicates were inoculated with 10 to 100 CFU and growth turbidity scored using McFarland Standards using a scale from zero to four. The total scores were tabulated and averaged for each media tested.

Results: PVA media performed as well as the autoclaved media prepared from commercial dehydrated media for all nine formulations evaluated. The PVA media produced a total score of 138, with a 2.56 average per replicate. The two traditionally made medias produced total scores of 132 and 121 with average scores of 2.44 and 2.24, respectively.

Significance: The data generated within the evaluation indicated that the PVA media performed equivalent to current commercially available bulk dehydrated media and can provide the end user a high level of quality assurance and quicker time to results by eliminating the need for traditional media preparation steps.

P1-95 Molecular Characterization of Methicillin-resistant *Staphylococcus aureus* and the Discovery of Novel *Spa*-Types

SAEED KHAN[1], Kidon Sung[1], Jung-Whan Chon[1], Bernard Marasa[2] and Mohamed Nawaz[1]
[1]U.S. Food and Drug Administration/NCTR, Jefferson, AR, [2]U.S. Food and Drug Administration, CDER, Silver Spring, MD

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates pose a serious threat to public health and cause minor, serious, and life-threatening infections. Apart from being present in hospital (HA-MRSA) and community settings (CA-MRSA), MRSA from food-producing and companion animals has, also, been shown to cause infection in humans. In this study, we have compared pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and *spa* typing profiles of 19 Panton-Valentine leucocidin (PVL)-positive HA- and CA-MRSA isolates.

Purpose: In this study, we have determined the molecular diversity of six PVL-positive CA-MRSA and 13 HA-MRSA isolates with respect to their PFGE, MLST, SCC*mec*, and *spa* typing profiles.

Methods: The antimicrobial susceptibility and DNA fingerprinting profiles of MRSA isolates were used to determine the similarity index among MRSA isolates. PCR and sequencing was performed to study their MLST, SCC*mec*, and *spa* typing profiles. The results were used to assign and compare their MLST- and *spa*-types with entries in online databases.

Results: Most of the MRSA isolates exhibited resistance to other drugs, including erythromycin gentamicin, kanamycin, oxacillin, and tetracycline. PFGE analysis suggested 10 different pulsotypes. The *spa* typing indicated seven known *spa* and two novel *spa* types. The most prevalent *spa* type was t030. MLST analysis revealed eight known ST-types, with the most common being ST-239. The majority of the isolates possessed SCC*mec* type III, alone or in combination with SCC*mec*IV and V. No correlation between the PFGE patterns (ST- or spa-types) was observed.

Significance: The similarities between CA- and HA-MRSA isolates and the presence of novel *spa* types indicate an evolutionary trend among MRSA. The data has yielded new and interesting information that could be helpful in understanding the mechanism of evolution, risk assessment of the threats posed by these isolates, and in the development of strategies to manage and control *S. aureus* infections.

P1-96 Effects of Coffee Mucilage Extracts on the Growth of Bacteria Associated with Disease, Food Deterioration, and the Human Gut

CAROLINA CHAVES, Maria Laura Arias, César Rodriguez and Patricia Esquivel
Universidad de Costa Rica, San Jose, Costa Rica

Introduction: Chlorogenic acids present in coffee beverages, as well as other phenolic components of coffee extracts have been reported to show some level of antibacterial activity. There are still no reports describing the antibacterial activity of polyphenol extracts of coffee processing byproducts.

Purpose: This study was performed to determine the effect of polyphenol extracts from coffee processing byproductst on the in vitro growth of microorganisms relevant to the food industry.

Methods: Polyphenol extracts were obtained from the coffee mucilage, as a byproduct of coffee processing, using the modified methodology described by Mingo et al. (2016), based on extraction with ethanol 50% (v/v). Dilutions of the extracts, with initial concentrations between 711 and 740 mg/ml, were added to different population densities of: foodborne pathogens *Staphyolococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Salmonella* Typhimurium ATCCC 13311 , and *Listeria monocytogenes* SLCC 4013; of food deteriorating bacteria *Pseudomonas* ATCC 27853, *Alcaligenes* UCR 277, *Serratia* UCR 299, *Micrococcus luteus* ATCC 4698, and *Escherichia coli* ATCC 35150; and human gut bacteria *Lactobacillus acidophilus* CRL730, *Lactobacillus casei* CRL431, *Lactobacillus rhamnos*us ATCC 7469, and *Lactobacillus plantarum*ATCC 14917 (human gut bacteria) through plate diffusion. The bacterial growth, as indicated by colony formation, was visually inspected after 24 hours of incubation under aerobic atmosphere at 35°C.

Results: Growth inhibition was observed for *B. cereus* and *Micrococcus* sp. in extract concentrations ranging from 30 to 70 and 14 to 70 mg/ml, respectively. While, the strains of *Serratia* sp., *Pseudomonas* sp., *L. plantarum* and *L. rhamnosus* were not inhibited.

Significance: Though the mechanism of action of the extracts remains to be elucidated, this type of extract could be used in the pharmaceutical or food industry, as natural preservatives of products prone to contamination with low GC% gram-positive bacteria.

P1-97 Isolation and Characterization of *Lactobacillus parafarraginis* KU495926 Inhibiting Multidrug-resistant Gram-negative Bacteria

RACHELLE ALLEN-MCFARLANE and Broderick Eribo
Howard University, Washington, DC

❖ ***Developing Scientist Competitor***

Introduction: *Lactobacillus* species are well known to produce bacteriocins with narrow activity against most gram-positive bacteria but less so for gram-negative bacteria.

Purpose: The aim of the current study was to isolate and characterize a lactic acid bacterium exhibiting antimicrobial activity against gram-negative bacteria.

Methods: Sixty-eight lactic acid bacteria (LAB) isolates from commercial yogurt and cheese were initially screened for antimicrobial activities against three standard indicator organisms: *Staphylococcus aureus, Listeria monocytogenes* and *Escherichia coli* (O157:H7).

Results: Although most of the LAB isolates (93%) exhibited antimicrobial activity against the indicator organisms, only one representative isolate was selected for further characterization. This isolate was identified to be *Lactobacillus parafarraginis* by 16S rRNA, accession number KU495926. It was of interest to note that the isolate inhibited fourteen multidrug resistant (MDR) and extended spectrum beta-lactamase (ESBL) bacteria from clinical sources by spot and well diffusion assays. The minimum inhibitory concentration (MIC) of the lyophilized crude extract was ~20 mg/ml. The 14 isolates were *Escherichia coli* (5), *Pseudomonas aeruginosa* (2), *Acinetobacter baumannii/haemolyticus* (3), *Enterobacter aerogenes* (1), *Proteus mirabilis* (2) and *Klebsiella pneumoniae* (1). Further analyses of the crude extract by SDS-PAGE, fast perfusion liquid chromatography (FPLC) and PCR suggested that the inhibitory agent is a bacteriocin. The antimicrobial activity was associated with a protein band of ~ 75 kDa while the polymerase chain reaction (PCR) detected the gene (*sakT-β*) for sakacinT-β chain in the bacterial chromosome, but not the gene (*sakT-α*) for sakacinT-α chain.

Significance: The results of this study suggest that the bacteriocin produced by *Lactobacillus parafarraginis* KU495926 may have potential application in the preservation of fermented dairy products.

Poster

P1-98 Shedding of Foodborne Pathogens by Slaughtered Reindeer in Northern Finland

CLAUDIO ZWEIFEL[1], Sauli Laaksonen[2], Lisa Fierz[1], Nicole Cernela[1], Maria Fredriksson-Ahomaa[2] and Roger Stephan[1]
[1]*University of Zurich, Zurich, Switzerland,* [2]*University of Helsinki, Helsinki, Finland*

Introduction: Various food-producing animals were recognized, in recent years, as healthy carriers of bacterial pathogens causing human illness. In northern Finland, the husbandry of reindeer is a traditional livelihood with meat as the main product. However, microbiological data from reindeer are limited.

Purpose: The aim of this study was to determine the shedding of *Salmonella* spp., *Listeria monocytogenes*, *Yersinia* spp., and *Escherichia coli* O157 by semidomesticated Finish reindeer at slaughter.

Methods: Fecal samples from 470 slaughtered reindeer (between six and seven months old) were examined for *Salmonella* spp. (ISO 6579:2007-10), *L. monocytogenes* (ISO 11290-1:2005-01), and *Yersinia* spp. (CIN agar). Moreover, samples were screened by real-time PCR (after enrichment) for Shiga toxin genes (*stx*) and, if positive, for the serogroup O157. *Escherichia coli* O157 isolates were serotyped, characterized by multilocus sequence typing (MLST), and tested for sorbitol fermentation, *stx*1 and *stx*2 (and subtypes), *eae* (intimin), and *hlyA* (hemolysin).

Results: *Salmonella* spp., *L. monocytogenes*, *Yersinia* spp., and *stx* genes were detected in 0, 3, 10, and 33% of the samples, respectively. The 15 *L. monocytogenes* isolates belonged to serotypes 1/2a and 4b. The 46 *Yersinia* spp. isolates were, mainly, identified as *Yersinia kristensenii* and *Yersinia enterocolitica*. Of the 153 *stx*-positive samples, 20% tested positive for the serogroup O157. The 25 obtained *E. coli* O157:H$^-$ (nonmotile, but *fliC*H7-positive) isolates belonged to the MLST sequence type 11. Twenty-four *E. coli* O157:H$^-$ isolates, which originated from one geographic area, did not ferment sorbitol and harbored *stx*1a, *stx*2c, *eae*, and *hlyA*. The sorbitol-fermenting *E. coli* O157:H$^-$ isolate (*stx*$^-$, *eae*$^+$, *hlyA*$^+$, *sfpA*$^+$) closely resembled a strain having lost *stx*.

Significance: Semidomesticated reindeer might be carriers of bacterial foodborne pathogens and constitute a so far little noticed reservoir for *E. coli* O157:H7/H$^-$. Strict compliance with good practices of slaughter hygiene is, therefore, crucial to prevent foodborne pathogens from entering the food chain.

P1-99 Isolation and Characterization of *Bacillus* spp. as Potential Probiotics for Poultry

Alejandro Penaloza-Vazquez[1], **LI MA**[2], Brienna Mileson[1] and Patricia Rayas-Duarte[1]
[1]*Oklahoma State University, Department of Biochemistry and Molecular Biology, Stillwater, OK,* [2]*National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Stillwater, OK*

Introduction: Probiotics have become one of the potential solutions to global restriction on antibiotic use in food animal production. Endospore-forming bacterial species has been attractive probiotics due to their long-term stability during storage.

Purpose: The objectives of this study were to isolate and characterize endospore-forming bacteria from natural environments as potential probiotics for poultry.

Methods: Sourdough and the gastrointestinal (GI) contents of young broiler chicks were used for the isolation of potential probiotics after preenrichment for endospore forming bacteria. The isolates were screened for their tolerance to acid and bile salt, production of phytase, alpha-amylase, protease, cellulolytic activity, and sporulation capacity. Selected isolates were further identified by 16S rRNA gene sequencing and screened for biofilm formation, compatibility with each other and antagonistic effects against three poultry pathogens including pathogenic *Escherichia coli, Salmonella* Muenchen, and *Enterococcus cecorum*.

Results: More than 200 endospore-forming bacteria isolates were recovered from both sources. Based on the screening tests, 47 isolates were selected and identified by 16S rRNA gene sequencing. Six strains were further selected based on their ability to form biofilm and antagonistic effects against poultry pathogenic bacteria. The selected strains were *Bacillus amyloliquefaciens* and *Bacillus subtilis*.

Significance: The selected strains demonstrated remarkable potential to be used as probiotic for poultry to improve feed conversion and boost the host resistance to pathogenic bacteria.

P1-100 *Salmonella* Is Unlikely to Develop Resistance to Cold Plasma Treatment Based on RNA Sequencing Analysis

LI MA[1], Chris Timmons[2] and Kedar Pai[2]
[1]*National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK,* [2]*Plasma Bionics, Stillwater, OK*

Introduction: Cold atmospheric plasma treatment is emerging as a food surface decontamination alternative that inactivates bacterial pathogens through a multimodal mechanism that may result in limited bacterial resistance development.

Purpose: The purpose of this study was to compare the transcriptomic response of *Salmonella* cells to successive rounds of plasma treatment with surface dielectric barrier discharge (SDBD) actuators.

Methods: Culture suspensions of *Salmonella enterica* subsp. *enterica* serovar Enteritidis were spot inoculated onto sterile glass cover slips and treated with SDBD cold plasma actuators for four minutes at one cm for five successive times. The treated and untreated control samples were washed by vortexing in sterile peptone and the resulting wash fluid suspension was used for total RNA isolation, cell enumeration, and evaluation of bacterial inactivation. rRNA-depleted libraries of isolated RNA were sequenced and transcriptomic differences between control and treated samples were evaluated.

Results: Bacterial inactivation rates were not significantly different ($P \leq 0.01$) for all five treatments. Among 1,136 differentially expressed genes with fold changes greater than 1.50 ($P \leq 0.01$, FDR$\leq$0.05), 492 were up-regulated (1.50 to 6.06-fold) and 644 were downregulated (-1.50 to -241.14-fold). Downregulated genes were associated with nutrient uptake, osmoregulation, alternative carbon and nitrogen source utilization, transcription, translation, and DNA damage repair. The major upregulated genes included those encoding pathogenicity associated proteins. Rapid cell lysis and lipid peroxidation caused a reduction in overall cellular stress by alleviating nutrient, osmotic, and desiccation stress.

Significance: No significant differences in bacterial inactivation rates or differential gene expression were identified that could potentially lead to resistance development. Cold plasma treatment may be a viable technology for food decontamination.

P1-101 Bacterial Metabolites from Intra- and Inter-Species Influencing Thermotolerance: The Case of *Bacillus cereus* and *Geobacillus stearothermophilus*

Mayra Gomez-Govea[1], Santos Garcia[2] and **NORMA HEREDIA**[2]

[1]Unversidad Autonoma De Nuevo Leon, San Nicolas De Los Garza, Mexico, [2]Universidad Autonoma de Nuevo Leon, San Nicolas, Mexico

Introduction: Bacterial metabolites with communicative functions could provide protection against stress conditions to members of the same species. Yet, information remains limited about protection provided by metabolites in *Bacillus cereus* and inter-species.

Purpose: This study investigated the effect of extracellular compounds derived from heat shocked (HS) and non-HS cultures of *B. cereus* and *Geobacillus stearothermophilus* on the thermotolerance of non-HS vegetative and sporulating *B. cereus*.

Methods: Cultures of *B. cereus* and *G. stearothermophilus* were subjected to HS (42°C or 65°C, respectively, for 30 min) or non-HS treatments. Cells and supernatants were separated and mixed in a combined array, then exposed to 50°C for 60 min and viable cells determined. For spores, D-values (85 and 95°C) were evaluated after 120 h.

Results: In most cases, supernatants from HS *B. cereus* cultures added to non-HS *B. cereus* cells caused thermotolerance to increase (D_{50}: 12.2–51.9) in comparison to supernatants from non-HS cultures (D_{50}: 7.4–21.7). The addition of supernatants from HS and non-HS *G. stearothermophilus* cultures caused the thermotolerance of non-HS cells from *B. cereus* to decrease, initially (D_{50}: 3.7–7.1), and in most cases, to subsequently increase (D_{50}: 18–97.7). Most supernatants from sporulating *G. stearothermophilus* added to sporulating cells of *B. cereus* caused the thermotolerance of *B. cereus* 4810 spores to decline, whereas the thermotolerance of *B. cereus* 14579 increased.

Significance: This study clearly shows that metabolites in supernatants from either the same or different species (such as *G. stearothermophilus*) influence the thermotolerance of *B. cereus*.

P1-102 Comparison of Thermal D-Values of Nonproteolytic *Clostridium botulinum* and *Bacillus cereus* Spores

TRAVIS MORRISSEY[1], Viviana Loeza[2], Eduardo Patazca[2], Lindsay Halik[2], N. Rukma Reddy[1], Guy Skinner[1] and Kristin M. Schill[3]

[1]U.S. Food and Drug Administration, Bedford Park, IL, [2]IFSH, Bedford Park, IL, [3]U.S. Food and Drug Administration, Division of Food Processing Science and Technology, Bedford Park, IL

Introduction: Although the popularity of extended shelf-life refrigerated (ESL) foods is increasing, risk from nonproteolytic *Clostridium botulinum* is a concern in these products. It is critical to identify the true target pathogen in these products: nonproteolytic *Clostridium botulinum* or *Bacillus cereus*.

Purpose: This study was performed to compare thermal inactivation kinetics of spores from high pressure (HP) resistant nonproteolytic *C. botulinum* type B and F and thermally resistant *Bacillus cereus* spores suspended in ACES buffer (0.05M, pH 7.0).

Methods: Spores of nonproteolytic *C. botulinum* strains, Ham-B, Kap 9-B, and 610-F, were prepared using biphasic media. *Bacillus cereus* spores (F4430/73 and 9818) were prepared using a Nutrient Agar (NA) method. Spores diluted in ACES buffer (0.05M, pH 7) to 10^5-10^6 CFU/mL were heat sealed in sterile NMR tubes and subjected to temperatures of 80-91°C in an oil bath. *Clostridium botulinum* survivors were determined by a 5-tube MPN method using TPGY broth after incubation for 10 weeks and *B. cereus* survivors by plate count using NA after 48 hour incubation.

Results: Thermal D-values of HP resistant nonproteolytic *C. botulinum* strains and thermally resistant *B. cereus* strains decreased as process temperature increased from 80 to 91°C. Thermal D-values at 80°C for Ham-B, Kap 9-B, and 610-F were 5.15, 9.60, and 3.39 minutes, respectively, while *B. cereus* F4430/73 and 9818 were 369 and 371 minutes, respectively, at 80°C. D-values of the *C. botulinum* strains decreased to <1.0 minute at 87°C, while *B. cereus* F4430/73 and 9818 were 21.9 and 31.6 minutes, respectively, at 91°C.

Significance: Based on D-values, results indicate that thermally resistant *B. cereus* spores, although inactivated by high pressure in combination with heat, are much more resistant to heat alone than HP resistant nonproteolytic *C. botulinum* spores. The data may be useful for eventually identifying surrogate strains for process validation.

P1-103 Evaluation of Enzyme Effects and Characterization of Modes of Biofilm Formation in *Bacillus cereus*

EUN SEOB LIM[1] and Joo-Sung Kim[2]

[1]Korea University of Science and Technology, Department of Food Biotechnology, Daejeon, South Korea, [2]Korea Food Research Institute, Sung-nam-Si, South Korea

Introduction: *Bacillus cereus* can cause food spoilage and food poisoning. In the food industry, biofilm formation by bacteria is a major problem.

Purpose: The objective of this study was to evaluate enzymes for the reduction of biofilms and to characterize modes of formation in *B. cereus* biofilms.

Methods: The biofilms of two *B. cereus*strains, ATCC 10987 (BC87; reference strain) and a strain isolated from tatsoi green leaf (BC4), were screened for sensitivities to DNase I , two proteases, and three polysaccharidases using a crystal violet assay. Then, the compositions of extracellular DNA (eDNA), proteins, and carbohydrates in extracellular polymeric substances (EPS) were compared.

Results: In BC87, the biofilm formation was significantly inhibited in the presence of DNase I or cellulose (P<0.05) and no enzyme was able to degrade the preformed biofilms. In BC4, however, the biofilm formation was significantly inhibited by the presence of all the enzymes tested (P<0.05), and those enzymes degrade the preformed biofilms, except for amyloglucosidase. In the analysis of EPS, eDNA content of BC4 was 5-fold higher (29.5 µg) than BC87 (5.7 µg), total protein content of BC4 was 7-fold higher (147.0 µg) than BC87 (20.2 µg), and total carbohydrate contents were similar between BC4 (548.7 µg) and BC87 (425.8 µg).

Significance: Our results give insight into *B. cereus* biofilm formation and maintenance and indicate that enzyme treatment can be used as a strategy to control biofilms of *B. cereus*.

P1-104 Passage of *Campylobacter* spp. Subtypes through 0.45 and 0.65 µm Filters

MARK BERRANG, Richard Meinersmann and Nelson Cox

U.S. Department of Agriculture-ARS-USNPRC, Athens, GA

Introduction: A 0.45 or 0.65 µm filter can be used as a means to separate *Campylobacter* spp. from complex samples, allowing detection on solid plating media. It is unclear what percentage of cells in a *Campylobacter* spp. suspension pass through a filter and result in visible colonies.

Purpose: The objective of this study was to compare the number *Campylobacter* spp. cells detected by the filter method to those detected by direct plating and determine if the filter method can be used as a means to estimate cellular density of an unknown *Campylobacter* spp. in suspension.

Methods: Overnight liquid cultures of five subtypes of *Campylobacter jejuni* and five of *Campylobacter coli*, all originally detected in chicken samples, were used for this study. Each subtype was applied to Campy-cefex agar, directly, and after filtration through a 0.45 or 0.65 µm filter. All plates were

allowed to dry for 45 minutes, filters were removed, plates were incubated, and colonies were counted. The data is presented as log CFU/mL. Three replications were conducted for all 10 isolates.

Results: The mean recovery by direct plating was 8.1 log CFU/mL. Regardless of pore size, the overall mean number of *Campylobacter* spp. detected using the filter method was significantly less than direct plating ($P < 0.05$); 5.4 log CFU/mL by 0.45 µm and 5.5 with the 0.65 µm filter. When analyzed by subtype, significant differences ($P< 0.05$) in ability to travel through the filter were noted, ranging from 3.0 to 6.3 log CFU/mL.

Significance: The number of *Campylobacter* spp. cells that can pass through a filter and make a colony on underlying solid media is dependent on subtype. The filter method does not appear to be reliable for determination of actual numbers per mL of an unknown *Campylobacter*.

P1-105 Effect of Water Activity and Temperature on Growth and Ochratoxin A Production by *Aspergillus fresenii* and *Aspergillus sulphureus* on Niger Seeds

YUNG-CHEN HSU, Dawit Gizachew and W.T. Evert Ting
Purdue University Northwest, Hammond, IN

Undergraduate Student Award Competitor

Introduction: Niger seeds are used for extracting cooking oil and used as cattle and bird feed. When stored improperly, Niger seeds support mold growth and production of mycotoxins. Ochratoxin A (OTA) is a ubiquitous mycotoxin produced by *Aspergillus* and *Penicillium*species. It has been found in a variety of food and feed worldwide.

Purpose: The purpose of this study was to evaluate the effect of water activity and temperature on growth of *A. fresenii* and *A. sulphureus*on Niger seeds and resulting OTA production.

Methods: The two fungi were cultivated on Czapek Yeast Extract agar (CYA) individually at 30°C for 5 days. Sterilized ground Niger seeds (2.5g) with different water activity (a_w) values (0.98, 0.94, and 0.90) were placed in separate petri plates. Each plate was spot inoculated with 10 µl spore suspension containing10^4-10^5conidia. Plates were then sealed with parafilm and incubated in a closed glass jar at 20 or 30°C for 10 days. Growth on each plate was determined by measuring the area covered by mycelium. OTA production in each inoculated plate was determined using enzyme linked immunosorbent assay after 5 or 10 days of incubation.

Results: Niger seeds with a_w from 0.90 to 0.98 supported both fungal growth and OTA production at both 20 and 30°C. Highest growth rates of the two fungi were observed on seeds with 0.94 a_w and incubated at 30°C for 10 days. It was also found that OTA formed more rapidly on seeds incubated at 30°C than at 20°C. Seeds with 0.98 and 0.94 a_w yielded higher OTA concentrations than did seeds with 0.90 a_w.

Significance: The data collected from this study can be used to establish storage standards of Niger seeds.

P1-106 Reducing the Thermal Resistance of *Bacillus cereus* Spores

ASWATHI SONI, Phil Bremer, Indrawati Oey and Patrick Silcock Silcock
University of Otago, Dunedin, New Zealand

Introduction: *Bacillus cereus* is a Gram positive, endospore forming foodborne pathogen, which can survive pasteurization regimes. Strains of *B. cereus* can germinate and grow in foods held at low temperatures and produce toxins, which cause diarrhea and/or vomiting when the food is consumed. The application of wet heat at 121°C for five minutes (retorting) will eliminate spores, but this is not a feasible option for all the food products as it can result in significant denaturation of protein structures, a loss of functionality, and/or produce undesirable texture, color, or flavor changes.

Purpose: The purpose of this work was to understand factors influencing the germination of *B. cereus* spores and to develop hurdle strategies to inactivate germinating spores and newly developed vegetative cells.

Methods: The impact of chilled storage at 4°C for 6 days on *B. cereus* spores in phosphate buffer (pH 7.2, 50mM) was assessed based on their thermal resistance (D-values), structural changes (SEM), and viability. Viability was assessed by plating for total microbial number (TMN: vegetative cells plus spores) and spore number (SN). To determine SN, vegetative cells were inactivated by holding the suspension at 80°C for 15 min. Structural changes were compared with those during germination using SEM.

Results: TMN and SN remained constant over 6 days at 4°C, despite D-values at 88, 92, 96, and 100°C reducing by 13.9, 8.3, 0.2, and 0.1 min. SEM images suggested that structural changes were different from cortex lysis in germination but were contributing to the loss of heat resistance, indicating onset of germination.

Significance: A better understanding of the mechanisms controlling spore survival and germination may help in the development of hurdle strategies designed to eliminate spores from heat sensitive food products.

P1-107 Effect of Temperature on Mycelia Growth and Aflatoxin B1 Production of *Aspergillus flavus* and *Aspergillus parasiticus* on Niger Seeds

JUAN FRANCISCO HERNANDEZ, Francisco Hernandez, Dawit Gizachew and W.T. Evert Ting
Purdue University Northwest, Hammond, IN

Developing Scientist Competitor

Introduction: Aflatoxin (AFB_1) is produced by *Aspergillus flavus* and *A. parasiticus*, which contaminate crops before and after harvest. Consumption of AFB_1 causes both animal and human diseases including liver cancer. Niger seeds (*Guizotia abyssinica*) are commonly processed for cooking oil and noug cake (a byproduct from Niger seed oil factories) is commonly used in cattle feed. A previous study showed that cows fed with AFB_1 contaminated noug cake excrete aflatoxin M_1 in their milk. To prevent AFB_1 contamination in Niger seed oil and noug cake, it is critical to determine storage conditions that support growth and AFB_1 production of A. *flavus* and A. *parasiticus* in Niger seeds.

Purpose: The purpose of this study was to determine the effect of temperature on mycelia growth and AFB_1 production of *A. flavus* and *A. parasiticus* on Niger seeds after 5 and 10 days of incubation.

Methods: Individual plates containing ground Niger seeds, which were adjusted to water activity of 0.98, were inoculated with 10 µl (10^6 spore/ml) spore suspension of *A. flavus* NRRL: 6513 or *A. parasiticus* NRRL: 2999. Plates were incubated at 20, 30, and 35°C for a total of 10 days and mycelia growth on each plate was recorded daily. AFB_1 was quantified via indirect ELISA after incubation for 5 and 10 days.

Results: Incubation temperature significantly affected both mycelial growth and AFB_1 production of the two fungi. The maximum growth rates and AFB_1 production of the two fungi were observed at 35°C after 10 days of incubation.

Significance: The results collected from this study may be useful to establish a storage guideline for Niger seeds.

Poster

P1-108 Monitoring of Illegally Added Compounds and Drugs in Foods: Prohibited Ingredients

JUNGEUN LEE, Woogin Cho, Soyoung Won, Inseon Kim, Jaehee Hyun, Kyeongwook Kim, Jaei Kim and Wooseong Kim
Center for Food & Drug Analysis, Busan Regional Korea Food & Drug Administration, Busan, South Korea

Introduction: Illegally added compounds and drugs have been detected in general or functional foods. Especially, unknown compounds with the modified chemical structure similar to that of anti-impotence drugs, such as tadalafil, and anti-obesity drugs, such as sibutramine, were frequently detected in various foodstuffs.

Purpose: The purpose of this study was to monitor illegally added compounds and drugs in foods to ensure food safety.

Methods: We monitored 70 food items advertised as slimming products or sexual enhancement products in offline and online markets. Samples (1-10 g) were dissolved in methanol (MeOH) and sonicated for 30 min. The MeOH solvent was filtered using 0.2 micron syringe filter and, then, analysed for 73 illegal compounds using LC/PDA and confirmed with LC/MS/MS.

Results: Analyses of 73 illegal compounds including anti-impotence drugs, anti-obesity drugs, and their analogs were achieved using LC/PDA and confirmed with LC/MS/MS. Tadalafil, icariin, fluoxetine, or sibutramine was detected in 10 food items ranging from 21.20~218.64 mg/g, 0.15~0.23 mg/g, 30.71~52.72 mg/g, and 27.60 mg/g, respectively.These results indicated that sustainable monitoring of illegal compounds in various foodstuffs is required for the safety management.

Significance: The results of this study will contribute to reinforcement the food safety management system by preventing the distribution of foods containing illegal compounds.

P1-109 Revised EN ISO 22964: Evaluation of Granucult® and Chromocult® Culture Media for Pre-enrichment, Selective Enrichment, and Detection of *Cronobacter* spp.

Barbara Gerten, Michael Gampe, **ANDREAS BUBERT** and Lisa John
Merck KGaA, Darmstadt, Germany

Introduction: EN ISO 22964 has been revised into a full EN ISO standard with an extended scope to *Cronobacter sp*p. detection in food products for humans and feeding animals and environmental samples. A non-selective pre-enrichment step in BPW (Buffered Peptone Water) is followed by enrichment in selective medium CSB (Cronobacter Selective Broth) and plating out and identification on chromogenc CCI (Chromogenic Cronobacter Isolation) agar.

Purpose: Growth promotion and isolation of *Cronobacter spp.* and *Cronobacter*-related species were tested and confirmed with BPW, CSB and CCI agar, as described by EN ISO 22964:2017.

Methods: For growth promotion >15 *Cronobacter spp.* strains, >10 *Franconibacter spp.* and *3 Siccibacter spp.* strains, including type strains and wild isolates from food, environmental samples and *C. sakazakii*reference material spiked into the food matrix, were used for evaluating the media from the whole workflow.

Performance testing for the quality assuarance of the culture media for productivity selectivity and specificity were tested, as given by the EN ISO standard.

Results: All *Cronobacter spp.* and non-*Cronobacter* strains from pure strains resp. spiked reference material were able to be detected, following the method given by the revised standard: non-selective pre-enrichment in BPW, incubated between 34-38°C for 18 h ± 2h, followed by selective enrichment in CSB, incubated at 41,5°C ± 1°C for 24 ± 2h and plated out and identified on CCI agar, incubated at 41,5°C ± 1°C for 24 ± 2h. Productivity, selectivity and specificity of the media affected the performance, as specified by the standard.

Significance: For the tested Granucult® BPW, CSB and Chromocult® CCI agar, the results of this study indicate the applicability of the methods and criteria as given in the revised International Standard EN ISO 22964:2017 "Microbiology of the food chain - Horizontal method for the detection of *Cronobacter spp.*"

P1-110 Does Irradiation of Half Fraser Broth, in Pre-weighed Pouches for *Listeria monocytogenes* Enrichment, Impact Growth Promotion and Stability?

ANNE PRIGGE[1], Andreas Bubert[2], Regina Petrasch[2] and Michael Bülte[1]
[1]University of Giessen, Giessen, Germany, [2]Merck KGaA, Darmstadt, Germany

Introduction: Pre-weighed, irradiated, dehydrated culture media in pouches are becoming increasingly popular in food labs, due to time and cost saving aspects. Media in this format are gamma-irradiated at 10-20 kGy to avoid autoclaving. As irradiation may stress media ingredients the growth promotion and stability of Half Fraser broth, prepared by autoclaving and from pouches was compared.

Purpose: Selectivity and growth promotion of *Listeria monocytogenes* in autoclaved Granucult® and non-autoclaved, gamma-irradiated, Readybag® Half Fraser broths were tested to demonstrate that irradiation has no influence, including cold room storage of primary enriched food samples for up to three days, as permitted by the new EN ISO/DIS 11290-1:2014.

Methods: For growth promotion four *L. monocytogenes* strains were incubated in both media preparations for 22h and 26h and two food matrices (prawns, cheese) were spiked with two *L. monocytogenes* food isolates. A 72h cold room storage step was included. Results were confirmed by Singlepath®L'mono immunoassay. Prepared media were stored up to 96 hours at 5°C and 25°C. Productivity and selectivity were tested at different time points.

Results: Both media preparations provided similar growth rates with all four *L. monocytogenes* strains after 22h and 26 h incubation (inoculation 10 cfu/250ml, total 40 samples). The cfu/ml slightly increased during cold room storage as *Listeria* are able to grow at low temperatures. Low level spiking (1 cfu/25g, 40 samples) of cheese and prawns showed comparable results, as calculated by Fisher's extract test. Productivity and selectivity were acceptable up to 96 h at 5°C and 25°C, during stability studies.

Significance: Irradiation of Readybag® medium has no significant influence on the growth promotion of *Listeria*, as verified in food trials and after cold room storage, which is in accordance with EN ISO/DIS 11290-1:2014. The avoidance of autoclaving and a media storage option reduces the food testing process and test costs.

P1-111 FSMA Rules and EU Food Safety Regulations: Differences and Opportunities

CLAUDIO GALLOTTINI[1], Franco Rapetti[2] and Sara Trombetti[3]

[1]ITA Corporation, Miami, FL, [2]ESI - Euroservizi Impresa Srl, Torgiano, Italy, [3]CISRAD Srls, Roma, Italy

Introduction: The EU REG.CE N. 178/2002 and the United States Food Safety Modernization Act (FSMA) have revolutionized food safety in Europe and the U.S. Fifteen years after publication of the European Hygiene Package, many small businesses still struggle to adapt hygienic standard based preventive procedures. The Preventive Controls Rule has yet to be fully implemented in the U.S.; but required food safety plans that include a hazard analysis and preventive controls approaches will require strong management and record keeping procedures to ensure a chain of accountability.

Purpose: This study compared the European Hygiene Package and the Preventive Controls Rule for Human Foods and their implementation.

Methods: A sample of 180 Italian food companies were selected that were subject to European regulations, had registered as a food processing facility with the FDA for export to the U.S., and had a Preventive Controls qualified individual in place.

Results: A number of major differences were found between the two regulations. These differences included the design of the Hazard Analysis and Critical Control Points program, which is by production phase in Europe rather than by single ingredient or process point, as in the U.S. The approach to setting critical limits are, also, different (single point vs average values).

Significance: European food processing facilities face a number of new challenges in implementing food safety rules that take different approaches to achieving the same goal.

P1-112 A Survey Study of the Food Safety Management Systems of Colombian Food Exporters to the United States of America

NAYRA ALVARINO-MOLINA and Deivis Lujan-Rhenals

Universidad Pontificia Bolivariana, Monteria, Colombia

Introduction: Food safety management systems (FSMS) are fundamental to sell food products in international markets, especially to the United States. There are several options of FSMS that food industries can implement, but this decision is particularly crucial to succeed as food exporter from a developing country.

Purpose: The purpose of this study was to examine the profile and the improvements that food exporting Colombian companies made to their FSMS in order to break into the US markets and determine if the benefits created by the Free Trade Agreement (FTA) have motivated food companies to export.

Methods: An on-line survey was conducted among 113 (non-coffee) food exporting Colombian companies from PROCOLOMBIA's list, the official database of international sales for non-traditional goods and services in Colombia. Data was analyzed by using descriptive statistics to depict frequencies following methods of histograms, bar graphs and distribution tables.

Results: The total survey response rate was 35.4%. The most common FSMS implemented by Colombian food exporters was Good Manufacturing Practices (87.5%), followed by Hazard Analysis Critical Control Points (50%), but there was a tendency to adopt two or more FSMS. One of the most important conditions that had to be improved was food safety education; nevertheless, the most costly investment was in maintenance of buildings and equipment. Companies made improvements mostly by contracting with food safety consultants and benefits of FTA influenced only 20% of companies to export.

Significance: This study was the first one to evaluate FSMS among Colombian food exporters and show detailed information about conditions that were necessary to enhance the FSMS to comply food safety requirements in order to reach the US market. Results also indicate that FTA has motivated a low number of companies since most of them were already exporting to the US before this agreement took effect.

P1-113 An Overview of Food Safety Compliance and Technical Accreditation in the Welsh Food and Drink Manufacturing and Processing Industry

ELLEN W. EVANS[1], Leanne Ellis[1], Ann Marie Flinn[2], Jessica Lacey[1], Jamie Old[1], David Lloyd[1] and Helen Taylor[1]

[1]ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom, [2]Food Technology Centre, Coleg Menai, Anglesey, United Kingdom

Introduction: It is essential for food and drink manufacturing and processing businesses (FDMPBs) to have food-safety scheme compliance/technical accreditation (FSSC/TA) to ensure product safety, enable brand protection, and to evidence due diligence in compliance with UK food law. Currently, data detailing the FSSC/TA of FDMPBs in Wales are lacking.

Purpose: This study aimed to provide an in-depth overview of FDMPBs FSSC/TA in Wales, including the drivers, benefits, barriers, and support to enable Welsh FDMPBs to obtain/maintain FSSC/TA.

Methods: A mixed-methods research approach was utilized, including self-completed questionnaires by Welsh FDMPBs (n=103) to identify FSSC/TA, focus groups with FDMPBs and stakeholders (n=59) to identify barriers to obtaining/maintaining FSSC/TA, and self-completed-questionnaires (n=34) to identify required support.

Results: The majority of Welsh FDMPBs (60%) reported having FSSC/TA, including British Retail Consortium (BRC) (24%) and Safe and Local Supplier Approval (SALSA) (16%). Micro-FDMPBs (<10 employees/turnover <£250,000) were associated with lack of FSSC/TA (P<0.001). SALSA was most frequently found among small/medium-FDMPBs, and BRC was associated with large-FDMPBs (>250 employees/turnover >£50M) (P<0.001). It was determined that FDMPBs with FSSC/TA were significantly more likely (P<0.05) to supply wholesale, major retailers, food service, and the public sectors than those without FSSC/TA. Customer requirement was the key drivers for FSSC/TA. Identified benefits of FSSC/TA included supply chain security and business growth. Barriers to obtaining/maintaining FSSC/TA were broadly defined as, knowledge and skills, time, cost and resources, and communication and access to information. Support to overcome barriers included funding for training and advice, assistance with the implementation of food safety management systems, and pre-audits.

Significance: Significant associations were determined between sector, size, and accreditation. The research has identified the support required for Welsh FDMPBs to overcome the barriers to obtaining/maintaining FSSC/TA. Such data may inform the development of support mechanisms to enable increased uptake of FSSC/TA and accelerate food sector growth that will align with Welsh Government aspirations.

P1-114 Assessment of Video Observation to Evaluate Hand Hygiene Practices of Food Handlers in Food and Drink Manufacturing and Processing Businesses: A Feasibility Study

ELLEN W. EVANS and Elizabeth C. Redmond

ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom

Introduction: Food handler hand hygiene (HH) is a significant contributory factor to foodborne illness. HH is the most effective method for preventing cross-contamination. Although informative, food-safety cognitions are not indicative of actual practices and maybe subject to biases. Thus, observation of behaviour is required. During observations, researcher presence can increase reactivity, whereas video observation (VO) provides a more comprehensive analysis over a sustained period, where familiarity reduces reactivity. Previous VO research has assessed food-retail/catering food handler hygiene behaviours; however, this method of assessment has been under-utilized in food and drink manufacturing/processing business (FDMPB) environments.

Purpose: This investigation determined the feasibility of using VO to evaluate food handler HH practices in FDMPBs.

Methods: In-depth interviews with FDMPB managers/technical supervisors (*n*=11) identified HH protocols, training procedures, and acceptability of conducting VO to assess HH compliance. Twenty-four hours of footage was observed to evaluate HH compliance in a bakery. Detailed observations for HH opportunities were recorded, specifying hand decontamination component actions.

Results: FDMPBs had unique HH protocols with variable details. Interviews identified positive attitude towards using VO to assess HH compliance. Although FDMPBs had cameras recording activity, none had the resource/time to conduct frequent/structured observation of footage. Observational findings indicated that of 674 instances, when food handlers entered production, 70 failed to attempt HH practices. Of 604 attempts to implement HH practices, only 2% were compliant with FDMPB protocol. Although 78% utilized soap, only 45% wetted hands first. Less than half (42%) utilized sanitiser. Malpractices included drying hands on overalls (9%). HH duration ranged from 1s–69s (median 17s).

Significance: VO data provided an in-depth insight into HH compliance when entering production and, thus, illustrated a valuable and useful resource for FDMPBs. Extensive numbers of HH malpractices were observed that were contrary to FDMPB policy and may compromise food-safety during food production. The case study identified site-specific issues to inform the development of an intervention to improve HH practices.

P1-115 Development and Validation of a Comprehensive Index to Evaluate Food Safety at the Household Level in India

S.G.D.N. LAKSHMI REDDI[1], SubbaRao M. Gavaravarapu[2], Naveen R. Kumar[1], Vishunuvardhana, Rao M. M[3] and Sudershan R. Vemula[1]

[1]Food and Drug Toxicology Research Centre, National Institute of Nutrition (ICMR), Hyderabad, India, [2]Extension & Training Division, National Institute of Nutrition (ICMR), Hyderabad, India, [3]National Institute of Nutrition (ICMR), Division of Biostatistics, HYDERABAD, India

❖ ***Developing Scientist Competitor***

Introduction: In India, foods are usually processed and prepared at home. Most foodborne diseases emanate from homes due to poor knowledge, practices, and limited enabling assets. Food safety at the household level is now a public health priority. There is a need for rapid assessment tools to evaluate the food safety status at the household level.

Purpose: This study was undertaken to develop and validate a comprehensive index (considering knowledge, practices, and enabling assets) to assess the food safety status at the household level in India.

Methods: A cross-sectional study was conducted among primary home food preparers (*n*=400) in rural and urban (200 each) areas of Telangana, India. A 87 item comprehensive index questionnaire which covered knowledge, practices, and enabling-environment was developed and administered. Demographic profiles, food safety risk perceptions, and incidence of food/waterborne diseases were, also, collected. Weightages were assigned for responses. The maximum possible score combining knowledge, practices, and enabling-environment scores was 205. In addition, stored cooked food samples (100 samples of 100g each) of rice, lentils, vegetarian and non-vegetarian foods, drinking water (250 ml), and hand rinses from subjects were collected for microbiological analysis (USDA-BAM).

Results: The mean score of the comprehensive index was 124.9. Index scores were significantly associated with the presence of fecal coliforms, *Salmonella* spp. and *Bacillus cereus*in food samples and hygiene indicator organisms in drinking water. Eleven out of 87 index parameters were significantly associated ($P<0.05$) with pathogen risk contamination levels in foods. The optimal cut-off value for the pooled, 11 item food safety index (FSI) score was ~ 9 and found to have a sensitivity (77%), specificity (74%) and AUC (-0.808), which are acceptable.

Significance: Validated index is a simple and useful way to evaluate the food safety status at the household level and can be used for designing targeted food safety promotion measures.

P1-116 Application of a Novel Supercritical Carbon Dioxide (CO_2) Drying Process to Inactivate Foodborne Pathogens on Cilantro and Strawberry

Siméon Bourdoux[1], Stijn De Sutter[1], Sara Spilimbergo[2], Alessandro Zambon[2], Filippo Michelino[2], Mieke Uyttendaele[1], Frank Devlieghere[1] and **ANDREJA RAJKOVIC**[1]

[1]Ghent University, Ghent, Belgium, [2]University of Padova, Department of Industrial Engineering, Padova, Italy

Introduction: Dried foods have been consumed as early as 20 000 BC and are nowadays more and more popular amongst consumers. However, little is known about pathogens survivability through drying processes. Despite a long shelf-life and a good stability over time, dried foods may still host pathogenic microorganisms waiting for an opportunity to develop upon rehydration.

Purpose: This study aimed to demonstrate that supercritical CO_2 drying could be used as a drying process to obtain safe dried foods.

Methods: Fresh cilantro and strawberries, inoculated with 3 strains-cocktails of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *S.* Thompson, or *Listeria monocytogenes*, were treated with pure supercritical CO_2. The following conditions were applied: 1) pressurization to 80 bar at 35°C immediately followed by depressurization, 2) pressurization to 100 bar at 40°C immediately followed by depressurization and 3) pressurization to 80 bar and 35°C followed by depressurization after 150 min. The depressurization rate was kept at 5 bar/min. Enumeration of the different strains was performed by standard plate count method. Experiments were performed in triplicate.

Results: When exposed to pressurization/depressurization only, the products lost less than 10% of their initial weight. After 150 min at 80 bar/35°C, the average mass loss was 88.7%. On strawberry, independent of the type of treatments, *E. coli* O157:H7 and *Salmonella* strains were reduced by 2 to 3 log units whereas *L. monocytogenes* strains were more susceptible with 4 log reduction. For coriander 4 to 6 log inactivation of *E. coli* O157:H7 and *Salmonella* strains was noted. *L. monocytogenes* strains were found to be more resistant showing only 4 log reduction.

Significance: These results indicates that supercritical CO_2 can be used for drying and for microorganisms reduction in a single process. However, the effect of the food matrix strongly influenced the behavior of the different microorganisms during the process.

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P1-117 Inactivation of Murine Norovirus and Bacteriophage MS2 on Strawberries and Blueberries by High Pressure Processing

MU YE, Yingyi Zhang, Catherine Rolfe and Alvin Lee
Illinois Institute of Technology/IFSH, Bedford Park, IL

Introduction: Multiple outbreaks associated with foodborne viruses occurred in recent years, due to the consumption of contaminated berry products. High pressure processing (HPP) has been recognized as a nonthermal processing technology for the food industry, capable of inactivating viral pathogens while retaining the organoleptic quality.

Purpose: The objective of this study was to evaluate the application of HPP on the inactivation of murine norovirus (MNV-1) and bacteriophage MS2 on strawberries and blueberries.

Methods: Fresh strawberries and blueberries (25 g) were spot-inoculated with MNV-1 or MS2, at approximately five and seven log PFU/sample, respectively. Fresh and frozen berries were vacuum-sealed, packaged, and HPP treated at 250, 300, 350, 400, and 600 MPa for 3 minutes, with the initial temperature at 4ºC. After treatment, viruses were extracted and recovered from the samples and quantified by viral plaque assay.

Results: Fresh strawberries and blueberries were inoculated with MNV-1 at 4.7 ± 0.6 and 4.4 ± 0.5 log PFU/sample, respectively. Higher viral inactivation in strawberries was observed as pressure levels increased. At 400 MPa, greater than 4 log reductions were achieved in fresh and frozen strawberries. The inactivation of MNV-1 was significantly lower in fresh and frozen blueberries, with 1.9 ± 0.5 and 2.2 ± 1.2 log reductions at 600 MPa, respectively. MS2 showed high resistance towards HPP, with less than one log reduction observed.

Significance: Effective inactivation of MNV-1 is achievable with HPP treatment for strawberries and blueberries. These results suggest HPP is a promising technology to improve microbial quality while retaining sensorial characteristics of berries.

P1-118 Inactivation of *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* spp. on Strawberries by Pulsed Light

MU YE[1], Dandan Feng[1], Sophie Zuber[2] and Alvin Lee[1]
[1]Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, IL, [2]Nestlé Research Center, Lausanne, Switzerland

Introduction: Foodborne pathogens such as human norovirus, *Salmonella* spp.*, Listeria monocytogenes,* and *Escherichia coli* O157:H7 have been frequently associated with outbreaks in fresh produce. Pulsed light (PL) is a novel nonthermal technology for rapid inactivation of microorganisms on food surfaces, equipment, and food packaging materials.

Purpose: The objective of this study was to investigate the inactivation of *E. coli*, *L. monocytogenes* and *Salmonella* spp. on strawberries using PL.

Methods: Surfaces of fresh strawberries (25 g) were spot-inoculated with cocktails of *E. coli*, *L. monocytogenes* and *Salmonella* spp. Samples were treated with PL using Xenon Steripulse XL-3000TM system at a rate of 1.8, 3, and 100 Hz for 30, 60, 90, and 120 s at a distance of 10.2, 12.8, and 15.3 cm from the sample surface to the UV strobe. After treatment, samples were serially diluted with buffered peptone water, plated onto tryptic soy agar with 0.6% yeast extract (TSAYE), and overlaid with violet red bile agar, modified oxford agar, and xylose lysine desoxycholate for *E. coli. L. monocytogenes* and *Salmonella* spp., respectively.

Results: The initial inoculum level was approximately seven log CFU/g. For different time durations ranging from 30 to 120 s, various degrees of inactivation, from 1.0 ± 0.2 to 3.1 ± 0.2 log CFU/g, were observed for the three bacterial pathogens studied. For example, a 60s treatment at 1.8, 3, and 100 Hz achieved approximately 2 log reduction of bacteria on strawberries. Overall, no significant difference in the inactivation was observed at measured distances from the UV strobe to the sample surface (P<0.05).

Significance: The present study demonstrated that PL treatment was an effective intervention method to inactivate bacterial pathogens on strawberries. However, processing conditions need to be optimized to minimize the impact on the quality of berries.

P1-119 Heat Inactivation of Tulane Virus in Inoculated Spinach Contained in Vacuum Bags

Sukriti Ailavadi, Mark Morgan and **DORIS D'SOUZA**
University of Tennessee, Knoxville, TN

❖ ***Developing Scientist Competitor***

Introduction: Tulane virus (TV) is recognized as a cultivable surrogate for human norovirus (HNoV), the leading cause of gastroenteritis worldwide.

Purpose: The study objective was to determine the heat inactivation kinetics (D- and z-values) of TV in inoculated spinach within vacuum bags, using linear and Weibull models.

Methods: TV, (at 7.25 log plaque forming units (PFU)/ml) inoculated into frozen chopped spinach and sealed in vacuum bags, was heated at 50, 54, 58°C for up to 8 min in a circulating water bath. Survivors were eluted by polyethylene glycol precipitation and assayed using LLC-MK2 host cells in six-well plates. Treatment were replicated thrice and assayed in duplicate. Data were statistically analyzed using PROCREG and NLIN in SAS 9.4 for linear and Weibull models, respectively.

Results: D-values at 50±1°C (± standard error; come-up time=44.5s), 54±1°C (79.5s), and 58±1°C (168.7s) were 7.94±0.21 (R^2=0.6; RMSE=0.3), 4.09±0.04 (R^2=0.6; RMSE=0.7) and 1.43±0.02 min (R^2=0.81; RMSE=0.65), respectively, with a z-value of 10.74°C (R^2=0.98; RMSE=0.25) and 6-D values of 47.64, 24.54 and 8.58 min, respectively, using the linear model. The Weibull model showed $T_{d=1}$ of 4.89±0.02 (R^2=0.99; RMSE=0.15; α [scale parameter]=2.24; β=0.17, where β=shape parameter of >1 means survivors are damaged, =1 linear, <1 resistant survivors are present), 3.11±0.45 (R^2=0.83; RMSE=0.67; α=1.09; β=0.78) and 0.25±0.38 min (R^2=0.96; RMSE=0.61; α=0.04; β=0.43) and $T_{d=6}$ of 55.87, 32.05, and 15.94 min, for the same temperatures, respectively. Previous studies showed that hepatitis A virus had a D-value of 34.4 min at 50°C and z-value of 13.92°C when inoculated into spinach in vacuum bags. D-values for murine norovirus (MNV-1) and feline calicivirus were 14.57 and 17.39 min, respectively, in inoculated spinach in 2-ml glass vials. This indicates that TV is not as heat resistant as earlier tested cultivable, foodborne viral surrogates.

Significance: Alternate surrogates that mimic the heat inactivation behavior of HNoV in foods need to be explored to ensure adequate thermal processing to maintain food safety.

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P1-120 Chlorine Dioxide Gas for the Inactivation of Human Norovirus Genogroup II on Formica Coupons

Purni Wickramasinghe, Mark Morgan and **DORIS D'SOUZA**
University of Tennessee, Knoxville, TN

❖ ***Developing Scientist Competitor***

Introduction: Human noroviruses (HuNoV), particularly genogroup II (GII), are predominant causes of non-bacterial gastroenteritis worldwide. Tulane virus (TV) is a cultivable HuNoV surrogate used to determine HuNoV inactivation methods. Chlorine dioxide (ClO_2) gas, a strong oxidizer, is being researched to determine antiviral effects against foodborne viruses.

Purpose: The objective of this study was to determine the ability of ClO_2 gas to inactivate HuNoV GII and TV on formica surfaces at room temperature.

Methods: HuNoV GII and TV preconditioned on formica coupons for 15 min were treated with 4 and 8 mg/L ClO_2 gas for 0, 1, 3, 5, and 8 min at 25°C and ~85% relative humidity. Viruses were recovered using Tris-glycine-beef extract buffer. HuNoV GII was detected by RNA heat-release and SYBR Green I-based RT-PCR with previously published MON primers and TV was detected by plaque assays. Each experiment was carried out in duplicate and replicated thrice.

Results: HuNoV GII, at initial 6 log RT-PCR units, was reduced by 1, 2, and 3 logs (based on end-point dilution) with 4 mg/L ClO_2 gas. TV showed reductions of 1.16 log after 1 min and to non-detectable levels (≥5 log) after 3 min with the higher 8 mg/L ClO_2 gas concentration.

Significance: ClO_2 gas treatments show promise in preventing HuNoV GII transmission and outbreaks.

P1-121 Decontamination of Whole Cantaloupe Using Chlorite and Acid in a Sequential Application

CHENG-AN HWANG[1], Lihan Huang[2] and Vivian Chi-Hua Wu[3]
[1]U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, [2]U.S. Department of Agriculture-ARS, Wyndmoor, PA, [3]U.S. Department of Agriculture-ARS-WRRC, Albany, CA

Introduction: The net-like surfaces of cantaloupe are difficult to clean. Pathogens, harboring on the surfaces, may subsequently contaminate the edible fruit part during preparation of fresh cut products.

Purpose: A new decontamination method using sodium chlorite and hydrochloric acid, in a sequential application to form chlorine dioxide in situ on the surfaces of produce, has been shown to significantly reduce pathogens inoculated on cantaloupe rinds. This study further examined the efficacy of the chlorite concentrations for decontaminating whole cantaloupes.

Methods: Three 1x1 cm rind surfaces of whole cantaloupes were spot-inoculated with a 5-strain cocktail of *Salmonella* spp. or *Listeria monocytogenes*. Each inoculated cantaloupe was submerged in 3 L sodium chlorite solution (0.4, 0.8, 1.2, and 1.6%) for 5 min, dried for 30 min, submerged in 3 L 6 mM HCl for 5 min, and dried for 30 min. After treatments, the inoculated rind sections were removed for enumeration of *Salmonella* spp. and *L. monocytogenes* using XLT-4 and PALCAM agars, respectively.

Results: The initial populations of *Salmonella* spp. and *L. monocytogenes* on the rinds were approximately 5.8 log CFU/g. Treatments with 1.2 and 1.6% chlorite reduced 5.4-5.8 log of *Salmonella* spp. and 5.2-5.4 log of *L. monocytogenes;* whereas 0.4 and 0.8% chlorite reduced 3.2-4.4 log of the populations. Higher concentrations of chlorite caused higher reductions of both pathogens. *Salmonella* spp. were more susceptible to the treatments than *L. monocytogenes*. Results showed that sequential treatments using >1.2% chlorite and 6 mM HCl could achieve a >5 log reduction of *Salmonella* spp. and *L. monocytogenes* on whole cantaloupe surfaces.

Significance: The new sequential treatment may be used for surface decontamination of whole cantaloupes to improve the product's microbiological quality and safety.

P1-122 Application of High Pressure Processing on Fresh and Frozen Strawberries and Blueberries to Inactivate *Salmonella* spp. and *Enterococcus faecium*

Mu Ye[1], Alvin Lee[1], Yingyi Zhang[2], Mengyi Huang[1] and **CATHERINE ROLFE**[2]
[1]Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, IL, [2]Illinois Institute of Technology/IFSH, Bedford Park, IL

Introduction: Berries are a popularized fruit for their utility as an ingredient in multiple food products, both fresh and frozen. Recent outbreaks have raised concerns regarding the safety of these fruits, specifically when consumed fresh. High pressure processing (HPP) effectively inactivates bacterial contaminants with minimal sensorial changes, proving to be an applicable technology for use with fruits such as berries.

Purpose: The purpose of this study was to identify the efficiency of HPP to inactivate *Salmonella* spp. and *Enterococcus faecium* (commonly used surrogate for *Salmonella*) in strawberries and blueberries.

Methods: Fresh strawberries and blueberries were spot-inoculated with approximately 6 log CFU/g of *Salmonella* or *E. faecium*. Berries were vacuum-sealed in high barrier pouches, packaged (fresh or frozen) and treated at varying pressure levels (250, 300, 350, 400 and 600 MPa) for 3 minutes at an initial temperature of 4°C. Visual analyses were conducted pre- and post- HPP for sensory assessment. Enumeration was performed using non-selective tryptic soy agar with 0.6% yeast extract (TSAYE), followed by a xylose lysine desoxycholate (XLD) overlay for *Salmonella* and m-Enterococcus agar overlay for *E. faecium*.

Results: HPP inactivation of *Salmonella* in strawberries demonstrated gradual inactivation as pressure levels increased, with 5.8 ± 0.6 and 4.9 ± 0.9 log CFU/g reduction at 600 MPa for fresh and frozen, respectively. *Salmonella* inactivation in fresh and frozen blueberries demonstrated an increase in inactivation at 600 MPa, fresh blueberries had a log reduction of 4.3 ± 1.1 log CFU/g compared to frozen with 2.5 ± 0.2 log CFU/g. Surrogate microorganism, *E. faecium*, demonstrated resistance to HPP inactivation, with less than 1 log CFU/g reduction at 600 MPa.

Significance: These results suggest application of HPP on fresh and frozen berries is an effective method for the inactivation of *Salmonella,* however, *E. faceium* may not be an appropriate surrogate for *Salmonella* to assess HPP efficacy.

P1-123 Surface Pasteurization of Post-harvest Raw Whole Onions to Eliminate *Listeria* Contamination Prior to Further Processing.

MANISH ARYAL and Peter Muriana
Oklahoma State University, Stillwater, OK

❖ ***Developing Scientist Competitor***

Introduction: The 2011-2012 outbreak of *Listeria monocytogenes* associated with cantaloupe created a heightened awareness for potential *L. monocytogenes* contamination of vegetables and produce. Due to easy contamination from various environmental sources and the long term exposure prior

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to harvest, *L. monocytogenes* may establish biofilms on produce or post-harvest surfaces. Processing methods to eliminate *Listeria* on produce surfaces reduces subsequent contamination of handle/cut/sliced pieces during downstream further processing operations.

Purpose: In-plant validation of a flame oven for eliminating surface contamination of *L. monocytogenes* of raw onions destined for further processing.

Methods: A gas-fired flame oven was used to test for lethality and process effectiveness on native microflora of onions (APC, yeast and mold) and inoculated (nonpathogenic) *Listeria innocua* (i.e., cocktail of antibiotic resistant ATCC 33090, ATCC 33091, and ATCC 51742). Control onions were sampled without heat treatment while heat-treated onions were placed on a conveyer belt for transit through the gas flames (47 sec) and processed through the oven before being sampled. Process evaluation included microbial enumeration of pre- and post-process levels of organisms. For selective enumeration of *L. innocua*, plating was done on TSA containing rifamycin and streptomycin (both at 50µg/ml) while tryptic soy agar (TSA) and potato dextrose agar (PDA), were used for indigenous bacteria and yeasts/molds, respectively. The data obtained was analyzed using one way ANOVA ($P< 0.05$).

Results: The onions inoculated with *L. innocua*showed approximately 5-log reduction. Approximately 5-log reductions were also obtained for indigenous bacterial contamination and 4-log reduction of yeast and molds when compared to unprocessed onions ($P< 0.05$). The gas flame burnt off the outer paper-like layer of the onions without damaging the onion 'meaty' layers and hence reduced yield loss.

Significance: The gas flame-fired oven heat treatment could be an effective means of reducing transfer of *Listeria* spp. during subsequent slicing/dicing operations.

P1-124 Kinetic Inactivation of Foodborne Pathogens and Model Viruses in Milk Using UV-C Irradiation

DANIELLE GUNTER-WARD[1], Ankit Patras[1], Manreet Bhullar[1], Agnes Kilanzo-Nthenge[1], Bharat Pokharel[1] and Michael Sasges[2]

[1]Tennessee State University, Nashville, TN, [2]TrojanUV, London, ON, Canada

❖ ***Developing Scientist Competitor***

Introduction: Elimination of viral and bacterial contamination is of prime importance in food production processes. The persistence of foodborn pathogens in milk has become a critical public health concern. Due to its rich nutrient content, milk is prone to spoilage by many pathogenic bacteria and viruses. Ultraviolet (UV) light technology, is comparatively more cost-effective, efficient, and sustainable than thermal pasteurization.

Purpose: The efficiency of UV-C irradiation as a nonthermal processing method for milk was investigated. Milk inoculated with two surrogate viruses (MS2, T1UV), *Escherichia coli* (ATCC 25922), and two pathogenic bacteria (*Salmonella* Typhimurium*, Listeria monocytogenes*) was treated with UV-C irradiation.

Methods: Optical properties of the samples were measured using a spectrophotometer connected to a single integrating sphere. Reduced scattering and absorption coefficients were mathematically extracted and accounted for in the dose calculations. Biodosimetry techniques were used to calculate the reduction equivalent dose (RED). Milk was irradiated using a continuous-flow reactor with the fluid pumped around a central low-pressure mercury UV lamp (40 W) emitting at 254 nm wave-length; this system achieved good mixing and uniform fluence. A series of known UV doses (0 – 50 $mJ{\cdot}cm^{-2}$) were delivered to the samples, in triplicate, followed by duplicate plating and enumeration.

Results: The populations of MS2, T1UV, *E. coli, S.* Typhimurium*,* and *L. monocytogenes* were reduced by more than five $\log_{10}$. At the highest dose of $40mJ{\cdot}cm^{-2}$, our results showed that UV-C irradiation effectively inactivated viruses and pathogenic microbes in milk. The inactivation kinetics of all microorganisms were best described by log linear models with a low root mean squared error (RMSE) and higher coefficient of determination ($R^2>0.95$).

Significance: This study demonstrated that high levels of inactivation of pathogens can be achieved in milk (highly opaque fluid) and suggested significant potential for UV-C treatment of milk.

P1-125 Study of Inactivation Effect of *Cronobacter sakazakii* on Nonfat Milk Powder

DONGJIE CHEN

University of Minnesota, St Paul, MN

Introduction: Intense Pulsed light (IPL) represents an excellent alternative or complement to conventional thermal or chemical disinfection of bacteria, thus ensuring safe foods with satisfactory nutritional and organoleptic qualities.

Purpose: Sickness from *Cronobacter* had been rising recently and often affects babies and older adults, Therefore, the development of new rapid control technology to kill Cronobacter bacteria in infant dried powdered foods/formulas is warranted.

Methods: Set the vibratory feeder speed at vibratory power 25% (7.6 cm/s), 20% (5.1 cm/s), & 15%(2.5 cm/s) respectively, so that IPL treatments were performed for a total time at 10 s, 15 s, and 30 s, at the distances between quartz window and IPL chamber were ~10.8 & 16 cm.

Results: The preliminary results and simulations show more than 3 log reductions of Cronobacter sakazakii. Temperature and powder water activity may also affect IPL inactivation.

Significance: Firstly, although IPL has been tested for many foods, there is no continuous IPL system commercially available for disinfection of powdered foods. The researchers proposed a number of mechanisms to enable continuous IPL treatment of powdered foods. Secondly, the project takes a systematic approach to evaluate not only the engineering and microbiological aspects of the technology but also the impacts of the process on physical, chemical, nutritional, and sensory properties of the products being treated.

P1-126 Inactivation of *Clostridium sporogenes* Spores in Buffer and Coconut Water Using UV-C Irradiation

SUDHEER KUMAR YANNAM[1], Ankit Patras[1], Yvonne Myles[1] and Michael Sasges[2]

[1]Tennessee State University, Nashville, TN, [2]TrojanUV, London, ON, Canada

Introduction: *Clostridium sporogenes* is an anaerobic non-pathogenic spore forming bacteria. It is widely used as a surrogate for *Clostridium Botulinum* to validate sterilization processes for low-acid foods. Application of thermal processing methods can alter the nutritional content and organoleptic properties of the foods. Ultraviolet (UV) light technology is comparatively more cost-effective, efficient, and sustainable than thermal pasteurization. It acts directly on the nucleic acids of the target microbe and impedes the microbe's replication.

Purpose: This study was designed to assess the ability of UV irradiation to inactivate *Clostridium sporogenes* in coconut water, a highly opaque liquid food.

Methods: Coconut water was extracted from raw green coconuts. Optical properties of the coconut water were measured using a double-beam spectrophotometer. UV irradiation was applied to stirred samples of coconut water, using a collimated beam system operating at 253.7 nm wavelength. A series of known UV doses (0 - 80 $mJ.cm^{-2}$) was delivered to samples of coconut water that had been inoculated with the target microbes. Spores were cultured and counted to determine inactivation.

Results: UV-C irradiation effectively inactivated *Clostridium sporogenes* spores in buffer and coconut water. At the highest dose of 80 mJ·cm^{-2}, spores were inactivated by more than 4 log CFU/mL (R^2=0.99, P<0.05) in both fluids. The D_{10} value of Clostridium *sporogenes* was estimated to be 19.12 mJ·cm^{-2}. The observed sensitivity of the organisms agreed with literature values Overall, this work demonstrates that UV-C has a potential for inactivation of *Clostridium sporogenes* spores.

Significance: This study demonstrated that high levels of inactivation *spores* can be achieved in coconut water (highly opaque fluid), and suggested significant potential for UV-C treatment of a low acid beverages.

P1-127 Assessing the Efficacy of Microwave on the Inactivation of *Bacillus coagulans* Spores in Coconut Water

RAQUEL OM PINTO[1], Renata B Nascimento[2], Cynthia J Kunigk[2], Luiz Alberto Jermolovicius[2] and Mariza Landgraf[1]

[1]University of São Paulo, São Paulo, Brazil, [2]Maua Institute of Technology, Sao Caetano, Brazil

Introduction: The consumption of coconut water has increased in the past decades, due to its nutritional composition and rehydration capability. Consequently, new processing technologies have been developed in order to maintain the sensorial characteristics and nutritional properties of this product.

Purpose: This study aimed to assess the efficacy of microwaves on the inactivation of *Bacillus coagulans* spores, experimentally inoculated into coconut water and to compare its efficacy with conventional heating.

Methods: Experiments were conducted according to the Central Composite Designs (CCD) with four parameters: temperature, 83°C - 95°C; potency, 115 - 135 W; amount of mixture of citric and ascorbic acids added to the coconut water, 73 - 77 mg/100 mL; and the composition of these acids, ascorbic 20% - 30% and citric 67% - 80%. Approximately 1433 *B. coagulans* spores CCGB (LFB-FIOCRUZ, Rio de Janeiro, Brazil) were inoculated into 100 mL coconut water samples obtained from green fruits and previously acidified (ascorbic and citric acid). After being exposed to microwaves and conventional heating as per the conditions of the CCD central point (89°C, 125 W, and 75 mg of acids [25% ascorbic and 75% citric acid]) for 5, 10, 15, and 20 min, the surviving spores in the coconut water were enumerated.

Results: The average reductions of *B. coagulans* spores in the coconut water processed by microwave and conventional heating were 3.0±0.6 log CFU/mL and 3.7±0.8 log CFU/mL, respectively. Among the tested parameters, the temperature was the most relevant to the inactivation of this microorganism (P<0.05, Student's t-test). When exposed to microwaves for 5 min at 95°C (lowest time/highest temperature), a reduction of five log CFU/mL was obtained.

Significance: Microwaves have been used to heat foods in commercial sterilization processes. In this study, microwaves showed potential as a process for inactivating of *B. coagulans* spores in coconut water.

P1-128 Withdrawn

P1-129 Utilization of Bioindicators to Validate Thermal Processes: Case Study Example for Small Canning Processors

FELIX BARRON and Philip Pstrak

Clemson University, Clemson, SC

Introduction: Small canning processors have limited resources to validate designed thermal processes. There is also limited published literature demonstrating the use of bioindicators to validate microbial inactivaton of "soups" in retortable pouches. Small canning establishments would benefit to know their new product/process can be assured of effective commercial sterilization by observing actual microbial inactivation by using bioindicators.

Purpose: The purpose of this study is to evaluate commercially available bioindicators to validate thermal processes and to demonstrate how a validation can be done by using a case study in a particulate food product such as a soup

Methods: At least 5 retortable pouches containing soup were subjected to a thermal process at 250F in a steam retort. The thermal process was repeated at least 3 times in order to determine the process time to achieve an Fo of at least 5 min. The thermal process is to be validated by using bioindicators (Geobacillus stearothermopilus 7953 (MesaLabs supplier) by follwoing recommended FDA and MesaLabs instructions.

Results: A minimum process time of 30 min at 250F is necessary to achieve the minimum lethality equivalent to an Fo of 5 min.

Significance: the use of bioindicators is a proven method to validate thermal processes; however, there is a lack of available literature to small canners informing them about the use of bioindicators to demonstrate the safety of their products has been achieved. The case study will be published in a format readily available to small canners including some form of an Extension or Public Service publication.

P1-130 Fabrication of Nano-engineered Stainless Steel to Prevent Biofilm Formation by Foodborne Pathogens

GAHEE BAN, Jaclyn Lee, Yong Li and Soojin Jun

University of Hawaii, Honolulu, HI

Introduction: Biofilms, the surface-associated complex communities of bacteria, adhered to both biotic and abiotic surfaces, have been explored in omnipresent environments. In particular, surface structure and related nano/microscale fabrication technologies have been intensively studied for prevention of microbial adhesion and consequent biofilm formation.

Purpose: This study demonstrated nanofabrication and anti-biofilm characterization of the self-cleanable surface on stainless steel substrates.

Methods: The 304 grade stainless steel surfaces were electrochemically etched in dilute Aqua Regia solution, consisting of 3.6% HCl and 1.2% HNO_3, at various voltages (5, 10, 15, and 20 V) and treatment times (5, 10, 15, and 20 min) to fabricate hierarchical nanoporous structures. Under static and dynamic flow environments, *Escherichia coli*, *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Pseudomonas aeruginosa* PAO1 were used for testing antibacterial adhesion and antibiofilm performances of the developed surfaces.

Results: Differences in applied voltage and treatment durations led to variations in the etch rate and surface morphologic characteristics. The plates treated at 10 and 15 V showed nanoscale pores, which are needed to improve the self-cleanability, while maintaining the intrinsic food grade quality of stainless steel. The etched samples coated with an additional hydrophobic Teflon layer showed a maximum static water contact angle above 150°. The surfaces etched at 15 V with Teflon coating, compared to the control stainless steel, showed significant antibacterial adhesion effects (P < 0.05) in both static and dynamic flow conditions.

Poster

Significance: The successful fabrication of superhydrophobic etched surfaces can be used in food industries to prevent biofilm development, resulting in the improvement of food safety.

P1-131 Application of Elevated Hydrostatic Pressure for Inactivation of Wild-type and Rifampicin-resistant Phenotypes of *Cronobacter sakazakii*

ELEONORA TROYANOVSKAYA, Abimbola Allison, Shahid Chowdhury and Aliyar Fouladkhah
Public Health Microbiology Laboratory, Tennessee State University, Nashville, TN

❖ ***Developing Scientist Competitor***

Introduction: Infections caused by *Cronobacter sakazakii* are often fatal to infants born premature, those younger than two months, and infants with weakened immune system. Application of emerging technologies could assure better control of this pathogen in the infant formula manufacturing environment.

Purpose: The current study investigated effects of elevated hydrostatic pressure on the cell reduction and inactivation rate of wild-type (WT) and spontaneous rifampicin-resistant (RR) phenotypes of *Cronobacter sakazakii*.

Methods: Various times (0 to 9 minutes) and intensity levels (0 to 380 MPa) of elevated hydrostatic pressure were investigated for inactivation of a 4-strain mixture of WT and RR *Cronobacter sakazakii,* inoculated into Tryptic Soy Broth (TSB) and TSB supplemented with rifampicin, respectively. The experiment was conducted at 4ºC, in two biologically independent repetitions, as blocking factors of a randomized complete block design, containing three repetitions per time/pressure/phenotype within each block. Study data was analyzed by the SAS generalized linear model for Tukey- and Dunnett-adjusted ANOVA. The inactivation K_{max}, 4D, and D-values were derived from the best-fitted (maximum R^2) model obtained by GInaFiT software.

Results: At 380 MPa (0 to 9 minutes), D-values of 1.73 and 1.40, 4D of 6.58 and 5.76, and inactivation K_{max} of 1.35±0.2 and 1.61±0.1 were observed for WT and RR phenotypes, respectively. Control counts of 7.54±0.5 and 7.58±0.3 log CFU/mL were reduced (P< 0.05) to 1.90±0.5 and <1.07±0.4 after 9 minutes of treatment at 380 MPa for WT and RR phenotypes, respectively. Treatments shorter than three minutes and below 240 MPa were less efficacious (P≥ 0.05) for reduction of the pathogen in vast majority of the tested intervals.

Significance: This study showed that greater than five-log *Cronobacter sakazakii* reductions are achievable, as the result of optimized high pressure pasteurization. The WT and RR phenotypes of the pathogen showed comparable sensitivity to pressure and inactivation rates and thus could be used interchangeably in microbiological challenge studies.

P1-132 Efficacy of Plasma Generated Novel Sanitizers in Egg Washing

SHRUTHI LAKSHMI NARASIMHAN[1], Shardul Dabir[1], Deepti Salvi[1], Donald W. Schaffner[2] and Mukund V. Karwe[1]
[1]Rutgers University, New Brunswick, NJ, [2]Rutgers University, Department of Food Science, New Brunswick, NJ

Introduction: *Salmonella* Enteritidis has been recognized as the primary cause of Salmonellosis associated with shell eggs and egg products. It has been associated with 60% of reported cases. FDA regulations require shell egg producers to control *Salmonella spp.* contamination of eggs. Chemical sanitizers, currently used, may have an undesirable environmental impact and can cause degradation of the egg cuticle, a natural defense mechanism against bacterial penetration into eggs.

Purpose: This project uses plasma activated water (PAW) and plasma treated acid electrolyzed water (PAEW) as novel and effective sanitizers for egg washing, to reduce microbial load with minimal loss of egg quality.

Methods: PAW and PAEW were generated by exposing distilled water or acid electrolyzed water to a cold atmospheric pressure plasma jet. The effectiveness of PAW, PAEW, and quaternary ammonium (QA) against a non-pathogenic *Salmonella* spp. surrogate (*Enterobacter aerogenes*) were evaluated in a planktonic-cell system. The novel sanitizers were, also, used to sanitize farm eggs, which were stained to detect cuticle loss by colorimetric analysis.

Results: Planktonic-cell experiments with distilled water caused reduction of 0.17±0.12 log CFU/ml from initial concentration of 9.21±0.06 log CFU/ml. Treatment with equal volumes of PAW, PAEW or QA resulted in a bacterial concentration below the detection limit (3.17 log CFU/ml). There was a statistically significant difference in microbial reduction between PAW/PAEW and distilled water (p-value <0.05), but no difference between PAW/PAEW and QA. A statistically significant difference was, also, noted by colorimetric analysis with respect to unsanitized eggs, between farm eggs treated with PAW/PAEW and store bought eggs. The DE-values observed were 13.98±5.51, 13.91±7.32, and 30.21±8.65 for PAW, PAEW, and industrially sanitized eggs, respectively. A lower DE-value suggested higher cuticle coverage.

Significance: PAW and PAEW appear to show promise for use in an egg washing process to replace current chemical sanitizers (QA) with minimal cuticle loss. Future experiments on eggs and with *Salmonella* spp. are needed.

P1-133 Antimicrobial Efficacy of Radiant Catalytic Ionization against Shiga Toxin-producing *Escherichia coli* on Inoculated Beef

XIANG YANG[1], Norasak Kalchayanand[2], Keith Belk[3] and Tommy Wheeler[2]
[1]University of California-Davis, Davis, CA, [2]U.S. Department of Agriculture-ARS, Clay Center, NE, [3]Colorado State University, Fort Collins, CO

Introduction: Shiga Toxin-producing *Escherichia coli* (STEC), as a common foodborne pathogen, may cause severe human hemorrhagic colitis infection. The radiant catalytic ionization (RCI) technology utilizes a combination of UV light and low-level oxidizers such as ozone, hydroxyl radicals, and hydrogen peroxide to cause antimicrobial action, which can be considered as a potential intervention to effectively control pathogen contamination on carcasses and resulting beef products.

Purpose: The purpose of the project was to evaluate the effect of RCI on reduction of selected *Escherichia coli* (*E. coli*) O157:H7, non-O157 STEC O26, O45, O103, O111, O121, and O145 strains.

Methods: Pre-rigor beef flanks (168 pieces; 5 ɔ5 cm; n = 6) were inoculated with one of seven inoculum groups to a final concentration of 5 to 6 log CFU/cm^2. The inoculated beef flanks were exposed to RCI treatment for 0 s (untreated control), 15 s, 30 s, or 60 s at 4°C. Samples were surface plated on SDA and brain heart infusion agar (BHA) using a spiral-plater and analyzed for survivors then compared with the untreated controls.

Results: The counts of survivor strains from treated samples were significantly (P < 0.05) lower compared to that of untreated controls. The reductions of selected STEC strains by 15 s RCI exposure ranged from 0.65 – 1.24 log CFU/cm^2. When the exposure time was increased from 15 s to 60 s, with current RCI settings, additional 0.45, 0.38 and 0.76 CFU/cm^2 reductions were detected on *E. coli* O26, O45 and O157, respectively. However, the counts of survivors for *E. coli* O103, O111, O121, and O145 remained unchanged (P > 0.05) even though the exposure time was increased to 60s.

Significance: This research provides preliminary data for proof of concept for antimicrobial efficacy of RCI against STEC on the surface of fresh beef.

Poster

P1-134 Evaluation of *Enterococcus Faecium* Nrrl B-2354 as Surrogate for *Salmonella* for Pasteurization Processes of Raisin

ERDOGAN CEYLAN[1], Yvette Avina[2] and Joe Leon[2]
[1]*Merieux NutriSciences, Crete, IL,* [2]*National Raisin Company, Fowler, CA*

Introduction: *Salmonella* is one of the major foodborne bacterial pathogens. It has been associated with outbreaks of low moisture products. Food facilities are required to implement and document that their preventive controls are working and verified. The use of pathogenic microorganism in plant environment may pose an unacceptable risk.

Purpose: The objective of this study was to evaluate *Enterococcus faecium* NRRL B-2354 as a potential thermal surrogate organism for *Salmonella* for in-plant validation studies of raisin.

Methods: *E. faecium* NRRL B-2354 and *Salmonella* were streaked onto Tryptic Soy Agar (TSA) and harvested using 0.1% peptone. Raisin samples (400 ± 1 g) were inoculated with 25 ml of the pooled inocula. Inoculated raisins were stored for up to 72 hr at 24 ± 2ºC to let them dry. Inoculated raisins were heat treated in an isotemp oven at 180°F.

Results: The thermal inactivation rates of *Salmonella* and *E. faecium* NRRL B-2354 were compared. The counts of *E. faecium* NRRL B-2354 were reduced by 0.6 log after 14 min and 3.3 log after 28 min at 180°F. The thermal reductions values for *Salmonella* were 1.4 log after 14 min and 6.1 log after 28 min at 180°F.

Significance: The data showed that *E. faecium* NRRL B-2354 was 1.4 times more heat resistant than *Salmonella* in raisin and could be used as a nonpathogenic surrogate for in-plant trials.

P1-135 Modeling the Effect of Thermal Stress on the Lag Phase of *Bacillus cereus* Strains in Reconstituted Infant Formulae

NATHÁLIA B. SILVA[1], Bruno A. M. Carciofi[1], Gláucia M. F. Aragão[1], Jozsef Baranyi[2] and Mariem Ellouze[3]
[1]*UFSC - Universidade Federal de Santa Catarina, Florianópolis, Brazil,* [2]*Imperial College, London, United Kingdom,* [3]*Nestlé, Lausanne, Switzerland*

❖ ***Developing Scientist Competitor***

Introduction: When cells are inoculated in a new environment, the specific growth rate is reached only after an adjustment period, called lag phase. It can be short or long depending on the pre-inoculation history of the cells. After going through a stressful condition, the adaptation period is longer than what they would need without pre-inoculation stress.

Purpose: In this talk we investigate the validity of the above conclusion, using *Bacillus cereus*as a model organism and heat treatment as a stress condition.

Methods: Three strains of *Bacillus cereus* were grown in RIF (Reconstituted Infant Formulae) at temperatures ranging from 12 to 25°C, after exposing them to thermal stress (72°C for 25 seconds). At 22°C also non-stressed cells were grown for control. Their lag times and specific growth rates were estimated using viable count measurements. The method of Analysis of Variance was used to study the variation of the parameter h_0, the product of the maximum specific growth rate and the lag.

Results: The heat treatment didn't affect the specific growth rates for none of the strains. For the strains B596 and F4810/72 grown in RIF, there was no significant difference between the h_0 values (P>0.1), but the strain B594 differs from the other two strains in this respect, too, just like regarding the growth rate (P<0.01). For the final predictive models, the h_0 value was taken as constant for each strain, estimated by the multiplicative average of its observed values (6.2, 4.1 and 4.9 for the strains B594, B596 and F4810/72, respectively). Finally, not surprisingly, the $h_0$value is low (ca 1) for the unheated cells, where the growth curves sometimes had hardly any lag.

Significance: Modelling the h_0 parameter leads to a simple way to model the effect of pre-inoculation history on the lag phase.

P1-136 Tracking Contamination through Ground Beef Production and Identifying Points of Recontamination Using a Novel Green Fluorescent Protein (GFP) Expressing, *Escherichia coli* O103, Non-pathogenic Surrogate

MICK BOSILEVAC[1], Brandon Luedtke[2], Rong Wang[3] and Yemi Ogunrinola[4]
[1]*U.S. Department of Agriculture–ARS, Clay Center, NE,* [2]*University of Nebraska at Kearney, Kearney, NE,* [3]*U.S. Department of Agriculture-ARS, Clay Center, NE,* [4]*Vantage Foods, Chilliwack, BC, Canada*

Introduction: Commonly, ground beef processors conduct studies to model contaminant flow through their production systems using surrogate organisms. Typical surrogate organisms may not behave as *Escherichia coli* O157:H7 during grinding and are not easy to detect at very low levels.

Purpose: Develop and validate a trackable *E. coli* O157:H7 surrogate, then model contamination passing through commercial grinding equipment using the surrogate.

Methods: The adherence, injury resistance, and recovery from low temperatures were determined for a non-virulent *E. coli* serogroup O103 constitutively expressing GFP (GFP-O103). Three 1,000lb (454Kg) combo-bins of beef trim were inoculated with 10^6 CFUs GFP-O103. Each inoculated combo-bin and four non-inoculated bins were sequentially used to produce 4lb (1.8Kg) ground beef loaves for monitoring GFP-O103 presence (n=90 tested per repetition).
Additionally, a single repetition of loaves prepared using 2,000lb (907Kg) combo-bins and initial inoculum of 10^4 CFUs was tested (n=178).
After each repetition, surface and residual meat samples were collected from processing equipment.

Results: The GFP-O103 was determined to be an ideal surrogate for *E. coli* O157:H7 and had a 1-5CFU/375g of ground beef limit of detection.
The grinding studies found contamination initially reached 0% prevalence in 10 sequential samples after a mean additional 2,480lb (1,125Kg) of non-inoculated material, however further sporadic positives indicated contamination persisted.
Regression analysis estimated that a total of 9,300lb (4,200Kg) non-inoculated material was required to attain a non-detectable level in our situation.
Potential recontamination points were identified on belts and augers where residual meat containing the GFP-O103 persisted through the end of production.

Significance: Disposition and recall decisions are based on data citing one 2,000lb (907Kg) combo-bin before and after an affected combo will clear contamination. Our results emphasize that each facility should perform their own studies due to unique line configurations and process complexities. The GFP-O103 is available for this use.

Poster

P1-137 Quantitative Microbiological Risk Assessment of *Campylobacter* spp. on Processed Ground Meat Products in S. Korea

JEEYEON LEE[1], Hanna Yoo[1], Heeyoung Lee[1], Ki Sun Yoon[2], Kun-Ho Seo[3] and Yohan Yoon[1]
[1]Sookmyung Women's University, Seoul, South Korea, [2]Kyung Hee University, Seoul, South Korea, [3]Konkuk University, Seoul, South Korea

Introduction: Recently, *Campylobacter* foodborne outbreaks have been increased, and associated with meat and processed meat products. In S. Korea, the consumption of processed meat products has been increased, thus, the risk of *Campylobacter* caused by processed meat products might be increased.

Purpose: The objective of this study was to evaluate the risk of campylobacteriosis through the consumption of processed ground meat products in S. Korea.

Methods: For exposure assessment, the prevalence of *Campylobacter* spp. in processed ground meat products such as cutlets, meat patties and meat balls was analyzed by plating the samples on modified CCDA-Preston. Conditions for storage and distribution for the products were surveyed by measurement and personal communication, and the collected data were fitted to @RISK program to obtain appropriate probabilistic distributions. Also, consumption patterns of processed ground meat products were investigated by a survey. Dose-response models for *Campylobacter* were investigated through literature review. Taken all data together, a simulation model was developed to estimate the risk of campylobacteriosis by the consumption of processed ground meat products.

Results: Of 224 processed ground meat products, the *Campylobacter* cell counts were detected under limit of detection (0.7 Log CFU/g). Thus, the initial contamination level of *Campylobacter* in processed ground meat products was estimated at -4.0±0.6 Log CFU/g. Means of storage and transportation temperature and time were -18.8°C (Pert Distribution) and 36-72 h (Pert Distribution). The survey data showed that one S. Korean consume 28.3 g (Exponential Distribution) of processed ground meat products per day at 6.98% frequency per day. Simulation result showed that the mean probability of *Campylobacter* foodborne illness caused by the intake of processed ground meat products per person per day was 5.68×10^{-10}.

Significance: The risk of *Campylobacter* in processed ground meat products is considered low in S. Korea, and results of this study should be useful in establishing the microbial guideline.

P1-138 Mathematical Model to Describe *Campylobacter* Survival in Processed Ground Meat Products

JEEYEON LEE[1], Hanna Yoo[1], Heeyoung Lee[2] and Yohan Yoon[1]
[1]Sookmyung Women's University, Seoul, South Korea, [2]Sookmyung Women's University, Seoul, Korea, Republic of (South)

Introduction: In recent, *Campylobacter* foodborne outbreaks have been increased and the outbreaks are usually caused by cross-contamination between raw meat and cooked meat products, which have a short shelf-life at cold temperature.

Purpose: The objective of this study was to develop the mathematical model to describe *Campylobacter* survival in processed ground meat products.

Methods: One tenth-milliliter aliquots of *C. jejuni* strains ATCC33560 and NCTC11168 were inoculated into 25-g of frozen pork cutlets (model food for processed ground meat products) to obtain 6-7 Log CFU/g. The samples were stored at 4°C, 10°C, 15°C, 25°C, and 30°C in aerobic condition, and *C. jejuni* surviving cell counts were determined by plating the samples on modified CCDA-Preston at appropriate time interval. The survival data were fitted by the Weibull model to determine δ (the time of first decimal reduction, h) and p (shape of curve). δ and p data were further analyzed by the Davey model as a function of storage temperature. The performance of the developed model was evaluated with the root mean square error (*RMSE*) by comparing predicted values to observed values.

Results: *C. jejuni* cell counts were decreased at all temperatures (4°C, 10°C, 15°C, 25°C, and 30°C). Low temperatures (4-15°C) showed longer ($P < 0.05$) δ values (37.2-94.2 h) than those (3.0-3.9 h) of high temperatures (25-30°C). p values were ranged from 0.5 to 1.2, and temperature effect on p values was observed. In addition, the secondary model (Davey model) of δ and p was appropriate with 0.890 and 0.910 of R^2. Validation result showed that the performance of developed model was appropriate with 0.589 of *RMSE*.

Significance: These results indicate that *C. jejuni* can survive in processed ground meat products for long time at lower temperature, and the developed model is appropriate to describe the kinetic behavior of *Campylobacter* in processed ground meat products.

P1-139 Mathematical Models to Describe Kinetic Behavior of *Campylobacter jejuni* in Dried Meat Products

JIMYEONG HA, Sejeong Kim and Yohan Yoon
Sookmyung Women's University, Seoul, South Korea

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Introduction: *Campylobacter jejuni* has been cause of foodborne outbreaks. Even though *C. jejuni* has susceptibility to various factors such as temperature, water activity and pH, the pathogen is found in raw meat as well as processed meat products. In addition, many studies indicated that low temperatures and vacuum packaging may increase *C. jejuni* outbreak.

Purpose: The objective of this study was to develop mathematical models to predict the kinetic behavior of *C. jejuni* in dried meat products.

Methods: Purchased seasoned and non-seasoned dried meat products were cut into 10-g portions. A mixture (0.1 mL) of *C. jejuni* strains ATCC33560 and NCTC11168 was inoculated into samples to obtain 5-6 Log CFU/g, and the samples were stored aerobically at 10°C, 20°C, 25°C, and 30°C. *C. jejuni* cell counts were enumerated on modified CCDA-Preston, and these results were used to develop a primary model (Weibull model) to calculate *Delta* (time required for first decimal reduction) and p (shape of curves). *Delta* values were further analyzed with a secondary model (Cubic model and Modified simple exponent model) as a function of storage temperature. To evaluate the accuracy of the model prediction, root mean square error (*RMSE*) was calculated by comparing the predicted data with observed data.

Results: In both seasoned and non-seasoned dried meat products, *C. jejuni* surviving cell counts gradually decreased at all temperatures. The *Delta* values from the primary model were longer ($P<0.05$) in 10-25°C than 30°C. No differences in p values were observed among temperatures. The secondary model well-described the temperature effect on *Delta* with 0.972-0.983 of R^2. *RMSE* values with 0.643 (seasoned jerky) and 0.519 (non-seasoned jerky) suggested that the model performance was acceptable.

Significance: This result suggests that the developed models should be useful in describing the kinetic behavior of *C. jejuni* in dried meat products.

Poster

P1-140 Quantitative Microbial Risk Assessment of *Campylobacter* spp. on Various Jerky

JIMYEONG HA[1], Sejeong Kim[1], Ki Sun Yoon[2] and Yohan Yoon[1]
[1]*Sookmyung Women's University, Seoul, South Korea,* [2]*Kyung Hee University, Seoul, South Korea*

❖ ***Developing Scientist Competitor***

Introduction: *Campylobacter* spp. have been well-known to cause campylobacteriosis in human. Although *Campylobacter* cell counts gradually decrease under various stresses such as oxygen and low a_w, the pathogen can cause foodborne illness because the minimum infectious dose of *Campylobacter* is low. In recent, the consumption of low-a_w food such as jerky has been increased in S. Korea.

Purpose: The objective of this study was to estimate the risk of *Campylobacter* spp. on various jerky in S. Korea.

Methods: For exposure assessment, the prevalence of *Campylobacter* spp. in beef and poultry jerky were investigated by plating the samples on modified CCDA-Preston. Distribution temperature and time for jerky were also surveyed. To predict the fate of the pathogen in the jerky under the distribution and storage conditions, the developed predictive models were cited. Consumption amount and frequency for the jerky were obtained by a survey. *Campylobacter* prevalence, distribution temperature, distribution time, consumption amount, and consumption frequency data were fitted to @RISK fitting program to obtain appropriate probabilistic distributions. Dose-response models for *Campylobacter* were searched through literatures. Eventually, a simulation model with @RISK were developed using collected data to estimate the risk of *Campylobacter* foodborne illness by intake of jerky.

Results: Of 250 jerky samples, there were no positive samples, and thus, initial contamination level was statistically predicted with RiskUniform distribution. The developed predictive models with Weibull model (primary model) and Cubic model (secondary model) were cited. Mean for jerky consumption amount was 51.833 g/day (BetaGeneral distribution) with 0.61% of frequency. The developed simulation model with all probabilistic distributions, predictive model, and dose-response model (Beta Poisson model) showed that the risk of *Campylobacter* foodborne illness per day per person was 8.53×10^{-10} for jerky.

Significance: These results suggest that risk of *Campylobacter* spp. in jerky could be considered low in S. Korea.

P1-141 A Novel Mathematical Model to Study Antimicrobial Interactions against *Campylobacter* spp.

MOHAMMED HAKEEM, Khalid Asseri and Xiaonan Lu
University of British Columbia, Vancouver, BC, Canada

❖ ***Developing Scientist Competitor***

Introduction: *Campylobacter* spp. is one of the leading causes of foodborne illness worldwide. In Canada, it is estimated that *Campylobacter* bacteria cause ~145,350 cases of foodborne illness per year. Thus, there is an urgent need to develop a new generation of antimicrobials to reduce the high numbers of *Campylobacter*-contaminated food products. Development of synergistic antimicrobial treatment can reduce the use of individual antimicrobial and maximize antimicrobial effect.

Purpose: In this investigation we searched for antimicrobial synergy against *Campylobacter jejuni.*

Methods: We compared the interaction of binary or tertiary combinations of cinnamon oil, encapsulated curcumin, and zinc oxide nanoparticles (ZnO NPs) using three methods; the time-killing assay, fractional inhibitory concentration index (FICI) method, and a mathematical model.

Results: We identified that each antimicrobial had a unique dose response curve, validating the need for quantitative assessment of an accurate interaction. The time-killing assay overestimated a synergistic effect between antimicrobials, while FICI method could not detect an existing synergistic phenomena. Specifically, encapsulated curcumin showed a sharp sigmoidal dose-response curve and cinnamon oil and ZnO NPs had slightly different hyperbolic dose response curves. In contrast, a mathematical model was constructed to successfully study the interaction in each combination and identify an accurate and reliable additive line. Nonlinear isobologram analysis was performed to evaluate the synergy in combinations, and a median effect equation was applied to identify combinations of synergistic effect at different reduction levels.

We observed an additive effect between cinnamon oil and ZnO NPs. The combination of encapsulated curcumin and ZnO NPs, at the same potency level at MIC_{10}, resulted in 99.42% reduction of *Campylobacter* with individual antimicrobial reduction of 60.51% and 81.76%, respectively. Combining cinnamon oil and encapsulated curcumin at MIC_{10} resulted in 92.65% reduction in bacterial cell count. Both antimicrobials were reduced by 93.40% and 60.51%, respectively. Tertiary antimicrobial combinations at MIC_{25} reduced bacterial population over eight log CFU/mL.

Significance: This novel mathematical model could accurately study antimicrobial interactions against *Campylobacter* bacteria and provide an alternative strategy to develop new antimicrobial chemotherapy.

P1-142 Quantitative Risk Assessment of *Listeria monocytogenes* in Ready-to-Eat Fish Products

PETRA PASONEN[1], Jukka Ranta[1] and Pirkko Tuominen[2]
[1]*Finnish Food Safety Authority Evira, Risk Assessment Research Unit, Helsinki, Finland,* [2]*Finnish Food Safety Authority Evira, Helsinki, Finland*

Introduction: *Listeria monocytogenes* is a common foodborne bacterium capable of causing severe disease, especially in persons with impaired immunity. Risk assessment is needed to evaluate the best risk management options to prevent listeriosis cases. Using Bayesian methods, as risk assessment tools has been proven useful due to their flexibility and ability to handle missing and fragmentary data.

Purpose: The purpose of this study was to assess the risk caused by *L. monocytogenes* in RTE fish products.

Methods: A Bayesian model, based on the Markov chain Monte Carlo computation, was developed for assessing the exposure for two populations: susceptible (elderly) and reference (working-age population). The exposure assessment model utilized data from national food survey (*n*=2,038) and *L. monocytogenes* surveys (*n*=1,083). This model was combined with an existing growth model and a dose-response model to predict the changes in risk (measured as annual illness cases in population) caused by the inappropriate storage practices of consumers.

Results: A mean dose in one contaminated portion varied largely with different scenarios. In scenarios where no growth was predicted, the mean dose per portion was approximately two $\log_{10}$ cfu; whereas, scenarios with high refrigerator temperature and long storage period could elevate the mean dose to approximately seven $\log_{10}$ cfu. Accordingly, predicted mean annual illness cases ranged from no cases to approximately 5,000 cases (median 1,600 cases) in the elderly population of size 600,000 and 130 cases (median 50 cases) in the reference population of size 2.8 million.

Significance: Temperature abuse and a long storage period was found to have a major effect on the predictions of mean annual listeriosis cases in the elderly.

Poster

P1-143 Modeling the Growth of *Listeria monocytogenes* in Cooked Deli Turkey Breast as a Function of a Clean Label Antimicrobial, Product pH, Moisture, and Salt

SUBASH SHRESTHA[1], Oscar Esquivel[1], Russ Lanzarth[2] and Jerry Erdmann[2]
[1]*Cargill Inc., Wichita, KS,* [2]*DuPont Nutrition and Health, New Century, KS*

Introduction: The potential for growth of *Listeria monocytogenes* in ready-to-eat (RTE) deli meat products, during storage, limits the food safety shelf life of the products. The USDA FSIS compliance guidelines for controlling *L. monocytogenes* in post-lethality exposed RTE meat and poultry products directs no more than two log cfu/g growth of *L. monocytogenes* over the stated shelf life of the product. The growth is affected by several factors including product formulations, such as presence or absence of antimicrobials and their usage levels. The product physico-chemical composition such as pH, moisture, salt content, etc. also greatly impact the growth of *L. monocytogenes*.

Purpose: The objective of this study was to develop a predictive model for the growth of *L. monocytogenes* in RTE uncured deli turkey meat, as a function of a clean label antimicrobial and product pH, moisture, and salt.

Methods: A central composite response surface design was used to investigate the effects of varying levels of product pH, moisture, salt content, and a commercial clean label antimicrobial (culture dextrose and vinegar blend; CDVB) on the growth of *L. monocytogenes* in RTE uncured deli turkey. Thirty treatment combinations of pH (6.1 to 6.9), moisture (69 to 81%), salt (0.5 to 2.5%), and CDVB (0.50 to 2.25%), including star points, were evaluated to develop growth curves. Treatments were surface inoculated with three log cfu/g of a five strain *L. monocytogenes* cocktail, vacuum packaged, and stored at 4°C for up to 16 weeks. Populations of *L. monocytogenes* was enumerated every week until growth stationary phase was reached. The lag times and growth rates for each treatment were estimated.

Results: Both the lag time and growth rate of *L. monocytogenes* was significantly affected by the product pH and the usage level of CDVB.

Significance: The availability of a *L. monocytogenes* growth prediction model allows companies to expedite the product development process by at least three months in this fast moving consumer market.

P1-144 Microbiological Survey of Not-Ready-to-Eat Frozen Foods

WEI CHEN[1], Loralyn Ledenbach[2], Joseph Meyer[2], Kurt Deibel[2] and Wendy McMahon[1]
[1]*Merieux NutriSciences, Crete, IL,* [2]*Kraft Heinz Company, Glenview, IL*

Introduction: In recent years, a heightened number of recalls and outbreaks of *Listeria monocytogenes*, a potential environmental contaminant, have been associated with Not-Ready-To-Eat frozen foods such as frozen vegetables. This has raised public awareness of this organism, but little data is available on the prevalence of *Listeria* spp. in these types of products.

Purpose: To evaluate the prevalence of *Listeria* spp. in selected 3 categories of Not-Ready-To-Eat finished frozen products from four different regions of United States.

Methods: At least three (3) different brands of frozen potato products (hash browns, French fries and tater tots) and frozen vegetables (frozen peas, corns, carrots and mixed vegetables), as well as 10 different frozen entrees were purchased from grocers across 4 different regions of the US (Northeast, Midwest, South and West). Products were coded to ensure blind testing. Products were then sampled (25 g), enriched and analyzed for *Listeria* spp. using BioMerieux's VIDAS *Listeria* (LIS) assay. Enrichments providing suspect assay results were confirmed by streaking to MOX media and evaluating for typical colonies. Typical colonies were considered confirmatory for *Listeria* spp.

Results: A total of 144 analyses (36 frozen potato, 48 frozen vegetable, 60 frozen meals or their individual components) were performed and 13 samples (6 frozen potato products, 6 frozen vegetable products, 1 component of 1 frozen meal) were positive (9%) for *Listeria* spp. Positive samples were found from all four regions.

Significance: Because 9% of products tested were found positive for *Listeria* spp., this could indicate the need for the Not-Ready-To-Eat frozen food industry to further evaluate their production environments, with emphasis on the importance of cleaning and sanitizing. Additional intervention measures, along with proper cooking instructions for consumers, can also play a part to minimize potential risk in these types of products.

P1-145 Fates of *Clostridium perfringens* in Marinated Steamed Pig Trotter under Changing Temperatures

HYEMIN OH[1], Heeyoung Lee[1], Hyun Jung Kim[2] and Yohan Yoon[1]
[1]*Sookmyung Women's University, Seoul, South Korea,* [2]*Korea Food Research Institute, Gyeonggi, South Korea*

❖ ***Developing Scientist Competitor***

Introduction: *Clostridium perfringens* are anaerobic bacteria and produce heat-resistant spores. Because of these characteristics, *C. perfringens* foodborne outbreaks have been increased in ready-to-eat or ready-to-heat meat products, and there were recently *C. perfringens* outbreaks related to ready-to-heat marinated steamed pig trotter consumption.

Purpose: This study developed a dynamic model to describe the fates of *C. perfringens* in processed pig trotter as a function of temperature and storage time.

Methods: A endospore mixture of *C. perfringens* (NCCP10970, NCCP10846, NCCP10347, NCCP10858, NCCP15911, and NCCP15912) were inoculated in marinated steamed pig trotter at 4 log CFU/g. The inoculated samples were then stored at 10°C (8 days), 15°C (5 days), and 25°C (2 days). *C. perfringens* cell counts were enumerated on tryptose sulphite cycloserine agar in anaerobic condition during storage. The cell counts data were fitted to the Baranyi model (primary model) to calculate death rate (*DR*; log CFU/g/h) and shoulder period (*SP*; h). A polynomial equation (secondary model) was then fitted to *DR* and *SP* as a function of storage temperature, and a dynamic model was developed in accordance with primary and secondary models. The model performance was evaluated by root mean square error (*RMSE*), accuracy (*A*) factor, and bias (*B*) factor.

Results: From 10 to 20°C, *SP*s were rapidly decreased ($P<0.05$), and *DR*s were increased ($P<0.05$). Developed secondary models were appropriate to describe the effect of storage temperature on the kinetic parameters with 0.995 of R^2 for *SP*s and 0.993 of R^2 for *DR*s. In addition, the performances of developed models were appropriate with 0.527 of *RMSE*, 1.07 of *B* factor, and 1.08 of *A* factor. The prediction of developed dynamic model was also appropriate at changing temperatures.

Significance: These results indicate that the developed dynamic models should be useful in describing the kinetic behavior of *C. perfringens* in marinated steamed pig trotter during storage.

Poster

P1-146 Isolation and Serotyping of *Listeria monocytogenes* from Smoked Salmon, and Developing a Dynamic Model to Predict *L. monocytogenes* Survival in Smoked Salmon

HYEMIN OH[1], Hyun Jung Kim[2] and Yohan Yoon[1]

[1]Sookmyung Women's University, Seoul, South Korea, [2]Korea Food Research Institute, Gyeonggi, South Korea

❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* outbreaks have been associated with smoked salmon and smoked meat in many countries. Although *L. monocytogenes* outbreaks have not been reported in S. Korea, the pathogen has been recently isolated from smoked salmon.

Purpose: This study isolated and serotyped *L. monocytogenes* from smoked salmon, and developed a dynamic model to predict *L. monocytogenes* survival in smoked salmon.

Methods: Thirty two of smoked salmon samples from retail markets were plated on PALCAM agar. Isolated *L. monocytogenes* colonies were identified by 16s rRNA analysis, and presences of *hly*A and *prs* genes were investigated by PCR. Serotypes were also determined by multiplex PCR. To develop a dynamic model, *L. monocytogenes* cell counts were obtained from smoked salmon during aerobic storage at 4-20°C for 8 days. The Baranyi model (primary model) was fitted to the microbiological data to calculate maximum specific growth rate (μ_{max}; log CFU/g/h) and lag phase duration (*LPD;* h). Polynomial equations (secondary model) were then fitted to the kinetic parameters. A dynamic model was developed with primary and secondary models. Root mean square error (*RMSE*) was calculated to evaluate the model performance.

Results: Of 32 smoked salmon samples, one sample (3.1%) was contaminated with *L. monocytogenes*, and the serotype was determined to be 1/2a. At 4-20°C, *L. monocytogenes* cell counts in smoked salmon increased at 0.01-0.13 log CFU/g/h of μ_{max}. *LPD*s were decreased from 74.2 to 4.8 h as temperature increased. Developed secondary model was appropriate to describe the effect of storage temperature on the kinetic parameters with 0.973-0.990 of R^2. The performance of developed dynamic model was appropriate at changing temperature with 0.368 of *RMSE*.

Significance: The results indicate that *L. monocytogenes* is contaminated in smoked salmon in S. Korea, and the developed dynamic model should be useful in describing kinetic behavior of *L. monocytogenes*.

P1-147 Kinetic Behavior of *Escherichia coli* in Steamed Pig Feet

HYEMIN OH[1], Soomin Lee[1], Hyun Jung Kim[2] and Yohan Yoon[1]

[1]Sookmyung Women's University, Seoul, South Korea, [2]Korea Food Research Institute, Gyeonggi, South Korea

❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* have been isolated from various animal by-products. In recent, there are several foodborne outbreaks related to steamed pig feet. Hence, it is necessary to describe the kinetic behavior of *E. coli* in steamed pig feet during storage.

Purpose: This study developed a dynamic model to describe the kinetic behavior of *E. coli* in steamed pig feet at changing temperatures.

Methods: Prepared steamed pig feet samples were inoculated with a five-strain mixture of *E. coli* strains at 4 log CFU/g. The samples were stored at 10°C for 8 days, 20°C for 6 days, and 25°C for 6 days. Total bacterial and *E. coli* cell counts were enumerated on tryptic soy ager and 3M Petrifilm™, respectively. The Baranyi model was fitted to *E. coli* cell counts data to calculate growth rate (log CFU/g/h) and lag phase duration (*LPD*; h). The kinetic parameters were further analyzed by a polynomial equation to evaluate the effect of storage temperature, and a dynamic model was subsequently developed, using kinetic models at changing temperatures. The model performance was evaluated with accuracy (*A*) factor, bias (*B*) factor, and root mean square error (*RMSE*) values by comparing observed and predicted data.

Results: Total bacterial counts and *E. coli* cell counts were increased ($P<0.05$) in steamed pig feet samples at all storage temperatures (10-25°C). As storage temperature increased, *LPD* decreased ($P<0.05$), and growth rates increased ($P<0.05$). Secondary models were appropriate to describe the effect of storage temperature on the kinetic parameters with 0.897- 0.942 of R^2. The developed kinetic models showed good performance with 0.618 of *RMSE*, 1.02 of *B* factor and 1.08 of *A* factor, and the prediction of the dynamic model was also appropriate.

Significance: These results indicate that the developed dynamic model should be useful in describing the kinetic behavior of *E. coli* in steamed pig feet during storage.

P1-148 Quantitative Risk Model for Predicting *Mycobacterium avium* subsp. *paratuberculosis* Contamination in Bulk Tank Milk on Dairy Farms

SURABHI RANI[1] and Abani Pradhan[2]

[1]University of Maryland, Hyattsville, MD, [2]University of Maryland, College Park, MD

Introduction: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is a pathogenic bacterium associated with Johne's disease, one of the important endemic infectious diseases in dairy cattle. Contamination ny MAP can be through direct shedding in milk by cows (internal route), entering of feces in the bulk tank milk (fecal route), or via contact with soil and water containing MAP (external/environmental route). Considering that there are reports of its survival in milk after pasteurization, humans can be exposed to MAP via milk (raw or pasteurized) consumption.

Purpose: The goal of this study was to develop a quantitative risk model of MAP transmission on dairy farms, including all three aforementioned routes, with a focus on different infection statuses of the infected cattle.

Methods: A probabilistic modeling framework was used to predict the MAP contamination from cows' shedding (internal route), feces, surfaces of milking parlor, and other environmental sources into the bulk tank milk. The data were extracted from a survey on dairy farms across the U.S. and published literature.

Results: The model estimated the final concentration of MAP in bulk milk to be, on an average, 3.07 log CFU/L with a maximum of 25 log CFU/L. Average dirt contamination containing feces played the most significant role in contributing MAP contamination in bulk milk, followed by herd size. Washing practice efficiency contributed to lowering the total contamination.

Significance: This study emphasized that good hygiene practices and proper herd management are crucial for maintaining the quality of raw milk on a dairy farm.

P1-149 Growth and Enterotoxin Production of *Staphylococcus aureus* on Beef Jerky as a Function of Temperature

Gun Woo Nam[1], Yeon Ho Kim[1], **YUN JIN LEE**[1], Mi jin Kwon[1], Soo Hwan Seo[2], Jeong A Han[2] and Ki Sun Yoon[1]
[1]Kyung Hee University, Seoul, South Korea, [2]Food Microbiology Division, Ministry of Food and Drug Safety, Chungcheongbuk-do, South Korea

Introduction: The production and sales volume of various kinds of beef jerky are increasing in Korea. Thus, it is necessary to evaluate the microbiological safety of beef jerky at retail markets.

Purpose: The object of this study was to investigate the effect of temperature on the possibility of *Staphylococcus aureus* (ATCC 13565) growth and enterotoxin A (SEA) production on beef jerky with low water activity at retail markets.

Methods: Beef jerky without seasoning was purchased and inoculated with *S. aureus* producing enterotoxin A at a concentration of approximately 3-4 log CFU/g. The Gompertz model was used to obtain growth kinetics and the concentration of SEA was measured using a RIDASCREENSET Total Kit (R-biopharm, Darmstadt, Germany) at 19, 20, 21, 25, 30, and 35°C.

Results: The growth of *S. aureus* on beef jerky was observed at 21°C. As the temperature increased (21, 25, 30 and 35°C), the LT values decreased from 31.73 to 16.35 h, and the SGR and MPD values increased from 0.012 to 0.120 log CFU/g/h and from 4.9 to 7.5 log CFU/g, respectively. At 25 and 30°C, SEA was not produced on beef jerky, but the concentration of SEA reached 0.24 ng/ml (7.5 log CFU/g) after 77 h storage at 35°C.

Significance: Despite the low water activity (Aw) of beef jerky (0.754), the growth of *S. aureus* and SEA production was observed at 21°C and at 35°C, respectively. Thus, beef jerky must be stored temperature lower than 21°C at the market and home to prevent risk of foodborne illness due to *S. aureus* growth.

P1-150 Optimal Isothermal Data Collection Practices for Estimating Microbial Thermal Inactivation Parameters

IAN HILDEBRANDT and Bradley Marks
Michigan State University, East Lansing, MI

❖ ***Developing Scientist Competitor***

Introduction: Isothermal inactivation studies are utilized to quantify pathogen thermal resistance. Isothermal survival data are used to test treatment effects or estimate inactivation model parameters. Biological variability, data collection practices, and regression methodologies can impact the quality of prediction models. While information for minimal collection practices to estimate model parameters exists, no such guidelines for optimal practices are known.

Purpose: The goal was to quantify effects of data collection and regression practices on estimation of inactivation model parameters using simulated isothermal experiments.

Methods: Using MATLAB, synthetic isothermal inactivation data were generated assuming a true response (log-linear or Weibull models with a Bigelow-type secondary model), with random error, taking into account data collection variables (subsamples, replications, come-up-time (CUT)). Monte Carlo simulations were used to generate data for 1000 synthetic studies, each encompassing a defined number of observations, subsamples, and replications. Log-linear and Weibull models were fit to the synthetic data, using nonlinear regression.

Results: For the log-linear model, regardless of instantaneous or non-zero CUT, there was negligible bias between estimated and true model parameters (<2%). For the Weibull model with instantaneous CUT, at least 2 subsamples or 4 replications were required to reduce relative bias below 5%. For the Weibull model and non-zero CUT, the estimated parameters were increasingly biased from the true model parameters as the model became more nonlinear. For example, the scale and shape parameters were different ($P < 0.05$) from the true values, with biases of 145 and 29%, respectively, when the shape parameter was 0.5. Overall, additional subsamples were more effective than additional observations or replications in reducing parameter variance and error.

Significance: When true bacterial response is log-linear, estimated model parameters are reliably accurate, and reproducibility is best improved with subsamples. When the true response is nonlinear, then typical isothermal experiments may not be adequate to accurately estimate inactivation parameters.

P1-151 An Alternative Risk Ranking Method Based on Log Transformation for Ranking Produce-hazard Pairs

MIN LI[1], Moez Sanaa[2], Barbara Kowalcyk[3], Kostas Koutsoumanis[4] and Arie Havelaar[1]
[1]University of Florida, Gainesville, FL, [2]ANSES, Maisons-Alfort, France, [3]RTI International, Research Triangle Park, NC, [4]Aristotle University of Thessaloníki, Thessaloníki, Greece

Introduction: Risk ranking approaches can help identify and prioritize foods and/or hazards that may pose greatest risks to public health. The use of semi-quantitative risk ranking methods are relatively simple and flexible, but could result in substantial loss of information and limited resolution.

Purpose: The study aimed to compare published semi-quantitative risk ranking approaches with an alternative quantitative approach that includes log transformation (either with or without binning) to score individual food-hazard pairs across the ranking criteria, using fresh produce as a model system.

Methods: Data from literature were used to define scoring bins for ranking criteria used in a published risk ranking model. 10,000 food-pathogen pairs were randomly generated from uniform distributions over realistic ranges of the criteria using standard risk assessment methods to define a reference set, and these random variables were then transformed and aggregated according to the different ranking methods. The semi-quantitative method used bins to assign each criterion to an arbitrarily defined number, and the alternative methods used log transformed risk scores on a scale between 0 and 1 with or without binning. Individual criteria scores were then summed to derive a final risk score for each produce-pathogen pair. The ranking methods were compared to the reference set using scattergrams and Kendall's rank correlation coefficient.

Results: The alternative quantitative methods had markedly higher correlation coefficients than those of the semi-quantitative method. The log transformation without binning provides the best ranking relative to the reference method, and the log-transformation with binning performs almost the same. The results indicated that use of a quantitative model allows for a higher resolution and reduction in the loss of information and better alignment with sound mathematical principles.

Significance: A fully quantitative risk ranking method provides a useful approach to prioritize produce-pathogen pairs and support risk-based decision making.

Poster

P1-152 The Health-related Economic Burden of Foodborne Illness from Meat and Poultry

ROBERT SCHARFF

The Ohio State University, Columbus, OH

Introduction: As Americans' taste for meat and poultry has grown over recent decades, so has their exposure to potentially harmful pathogens. In this study I present up-to-date estimates of the burden of illness attributable to consumption of meat and poultry products.

Purpose: This study uses published and original estimates of illness source attribution to assess the number and costs of illnesses associated with meat and poultry in the United States. National estimates are supplemented with commodity group estimates and burdens at the U.S. state level.

Methods: Outbreak and expert opinion source attribution data is integrated into a cost-of-illness study for meat and poultry-related foodborne illnesses in the United States and across the individual states. Monte Carlo analysis in @Risk7.5 is used to incorporate and characterize numerous sources of uncertainty. Two cost-of-illness models are used to assess costs.

Results: Illnesses from meat and poultry are responsible for 4.2 million illnesses (90% C.I. 2.8–6.2 million) from specified etiologies, resulting in health-related costs of $13.3 billion using the conservative cost-of-illness method (90% C.I. $7.8–$19.8 billion) and $31.0 billion using the enhanced method (90% C.I. $9.7–$58.2 billion). The large share of illnesses from *Campylobacter* spp. and *Salmonella* (nontyphoidal) makes poultry the largest single commodity source of illness; with 1.4 million illnesses (90% C.I. 0.7–2.0 million) and costs of $3.3 billion (90% C.I. $1.5–$6.6 billion) and $12.2 (90% C.I. $2.8–$29.7 billion) for the conservative and enhanced economic models respectively.

Significance: Meat and poultry continue to be a significant source of foodborne illness; imposing a substantial economic burden on consumers. The estimates presented here can be used to demonstrate the utility of maintaining a public health system that aims to manage these risks.

P1-153 A Statistical Model to Determine the Thermal Inactivation of Three Heat-resistant *Salmonella*e in Liquid Egg Yolk

JOSHUA GURTLER[1], Hans Allender[2] and Deana Jones[3]

[1]U.S. Department of Agriculture-ARS, Eastern Regional Research Center, Wyndmoor, PA, [2]U.S. Department of Agriculture-FSIS, Washington, DC, [3]U.S. Department of Agriculture–ARS, Athens, GA

Introduction: We previously reported the inactivation kinetics of salmonellae in liquid egg yolk, but subsequently determined that the three *Salmonella* used in the present study were more heat resistant than those previously tested. Hence, the present results report the most heat-resistant inactivation kinetics of any published in the literature for liquid egg yolk.

Purpose: A study was conducted to set forth a mathematical model for determining the thermal destruction of three heat-resistant strains of *Salmonella* in liquid egg yolk to effect a 7-log reduction of the bacterium.

Methods: Commercial liquid egg yolk was inoculated with the three most heat resistant strains of those tested in our laboratory, which included *S.* Enteritidis Pt8, strains C405 and C398 as well as *S.* Oranienburg DD2229. The glass capillary tube method was used to test thermal resistance of salmonellae at temperatures of 58, 60, 62, 64 and 66°C. Total solids, percent fat and apparent viscosity of the liquid egg yolk were assessed.

Results: The logistic 3P model was selected out of sixteen models tested based on R^2 values. Results indicated that D-values for these five temperatures (viz. 58, 60, 62, 64, and 66°C) were 4.96, 1.65, 0.65, 0.23, and 0.05 min, respectively, which is greater than that reported in the literature. Based on these results, a 5-log destruction of salmonellae would require 8.25 min at 60°C and a 7-log inactivation would necessitate 11.55 min. A calculator was developed, that allows the user to set up the pasteurization regimens with a temperature and time to achieve any desired level of *Salmonella* inactivation between the temperatures of 58 and 66°C and its corresponding uncertainty expressed by a 95% confidence interval.

Significance: These data may assist processors and regulators in designing pasteurization regimens suitable for the production of safe pasteurized liquid egg yolk, used in many ready-to-eat foods.

P1-154 Survival of 22 Avirulent Strains of *Escherichia coli* and *Salmonella* spp. in Crop Soil with 10% Fast-pyrolysis Switchgrass Biochar, to Validate Surrogate Bacteria

JOSHUA GURTLER[1], Akwasi Boateng[2], Manan Sharma[3], Trevor Suslow[4], Xuetong Fan[1] and Tony Jin[1]

[1]U.S. Department of Agriculture-ARS, Eastern Regional Research Center, Wyndmoor, PA, [2]U.S. Department of Agriculture–ARS, Wyndmoor, PA, [3]U.S. Department of Agriculture–ARS, Beltsville, MD, [4]University of California-Davis, Davis, CA

Introduction: We previously reported the increased inactivation of pathogenic *E. coli* O157:H7 in crop soil as affected by the presence of biochar (a form of black carbon formed by heating biomass under low-oxygen conditions).

Purpose: A study was conducted to evaluate survival of 22 strains of avirulent *E. coli* and *Salmonella* in crop soil with 10% fast-pyrolysis switchgrass biochar. The goal was to validate effective surrogate strains of bacteria to use in future studies.

Methods: Twenty-two, individual, 90 g soil samples were amended with 10% biochar and inoculated with 5 strains of non-toxigenic *E. coli* O157:H7, 10 strains of non-pathogenic *E. coli,* and 7 attenuated vaccine strains of *Salmonella,* respectively. Samples were held at 21°C for up to nine weeks and populations determined on antibiotic- and chromogen-supplemented Tryptic Soy Agar. Three strains of non-pathogenic *E. coli* were resistant to 50ppm rifampicin, and all other 19 strains were resistant to 100ppm nalidixic acid.

Results: Initial day 0 populations averaged 5.31 log CFU/g of soil. By 7 days, two salmonellae and four *E. coli* were undetectable by direct plating (minimum detection limit = 100 CFU/g), while 4 strains were still >4.1 log. Eight strains were undetectable by week three, while non-pathogenic *E. coli* strain TVS355 was still at 4.67 log CFU/g. Twelve strains were undetectable by week 7, including all non-toxigenic O157:H7. However, three strains of non-pathogenic *E. coli* (TVS-353, TVS-354, and TVS-355), were all >4.1 log. At nine weeks of storage, only 7 of 22 strains were still detectable by direct plating, including (log CFU/g in parenthesis) the non-pathogenic *E. coli* TVS-354 (4.22), TVS-355(3.36), TVS 353 (3.06), and the attenuated salmonellae ATCC-53467 (4.44), Chi8089 (3.38), Chi4096 (2.78), and Chi3985 (2.0).

Significance: These results identify suitable surrogate bacteria for use in future biochar-soil decontamination studies and, potentially, other evaluations predicting pathogen persistence in soils.

P1-155 Survival of Osmotically Adapted and Non-adapted *Salmonella* spp. in Bakery Products Containing Different Sweet Fillings

IFIGENEIA MAKARITI, Anastasia Kapetanakou, Eleftheria Nazou and Panagiotis Skandamis
Agricultural University of Athens, Athens, Greece

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Introduction: *Salmonella* spp, is known to survive under low moisture conditions. Bakery products such as cream-filled brioche products acquire a water activity of approximately 0.85, depending on the a_w of the filling and the baking they receive, and hence, may support survival of the pathogen.

Purpose: The study aims to assess the survival of osmotically adapted and non-adapted *Salmonella* spp. in creams and cream-filled brioche-like products.

Methods: Fillings (praline, biscuit) as well as whole brioche-like products (containing praline or biscuit filling), provided by the manufacturer, were inoculated with approximately 6 log CFU/g of osmotically adapted and non-adapted five-strain composite of *Salmonella* spp. (Typhimurium, Agona, Reading, Enteritidis) and stored aerobically (120mL screw-capped container) at 15, 20, and 30°C. Adaptation of *Salmonella* took place in creams (praline, biscuit) with a_w adjusted 0.88, by adding sterile water to the original filling (aw 0.80-0.82) and incubation at 37°C for 1 h. Survival of *Salmonella* was assessed at regular time intervals throughout storage using thin layer agar method (n=2×2)

Results: The lower the storage temperature the slower the inactivation of *Salmonella* spp., since the pathogen survived (4.0-4.5 log CFU/g) after 30 days at 15°C, while at 30°C *Salmonella* was reduced below the enumeration limit by the 20th day. *Salmonella* performed the same way in biscuit cream and biscuit-filled brioche, while higher survival was observed in praline cream than in the praline-filled brioche at 20°C requiring 48 and 77 days respectively to reach enumeration limit. Adaptation of *Salmonella* resulted in higher survival rates compared to non-adapted cells, phenomenon more evident in praline cream and biscuit-filled brioche (reaching population differences up to 2 log CFU/g).

Significance: Assessing the effect of previous adaptation of *Salmonella* spp. on its subsequent survival in brioche-like products and creams would contribute in development of more accurate predictive models regarding the behavior of the pathogen in bakery-confectionary products.

P1-156 Impact of Exceptional Situations Occurring Prior to Microbial Reduction Treatment on the Risk of Human Salmonellosis Arising from the Consumption of Pistachios in the United States: A Quantitative Assessment

GORDON DAVIDSON[1], Sofia Santillana Farakos[1], Regis Pouillot[2], Rhoma Johnson[1], Judith Spungen[1], Insook Son[1], Nathan Anderson[3] and Jane Van Doren[1]
[1]U.S. Food and Drug Administration, College Park, MD, [2]U.S. Food and Drug Administration, CFSAN, College Park, MD, [3]U.S. Food and Drug Administration, Bedford Park, IL

Introduction: Detection of *Salmonella* on pistachios demonstrates the need to quantify how various events impact the risk from consumption of this tree nut.

Purpose: To conduct a quantitative risk assessment of human salmonellosis arising from the consumption of pistachios in the U.S. and to evaluate the impact of exceptional situations occurring prior to microbial reduction treatment.

Methods: A baseline exposure assessment model was developed that includes *Salmonella* prevalence and levels on floater and sinker pistachios in silo storage, microbial reduction treatment (0-5 log CFU), *Salmonella* persistence during post-processing and retail storage, and consumption levels. Exceptional situations occurring prior to microbial reduction treatment, including increasing the prevalence and levels of *Salmonella* cross-contamination in a float tank, *Salmonella* growth due to a delay in drying hulled pistachios, and *Salmonella* recontamination by pests in the storage silos, were each modeled with a subsequent microbial reduction treatment (0-5 log CFU). A homogeneous distribution of the *Salmonella* cells was assumed. Risks per serving and per exceptional situation were calculated using Monte Carlo simulations developed in R using the *mc2d* package. Variability was set to 10,001 replicates and uncertainty dimension to 501 replicates; these were modeled independently.

Results: The baseline model predicted a 90% reduction in risk of salmonellosis for each log of microbial reduction treatment level applied. The predicted relative risk of illness per pistachio serving was higher in the modeled exceptional situations – *Salmonella* cross-contamination in a float tank (6.8 to 7.5-fold higher), *Salmonella* recontamination by pests in the silos (1.0 to 1.2-fold higher), and *Salmonella* growth due to a delay in drying (1.1 x 10^2 to 4.3 x 10^8-fold higher) – when compared to the baseline model.

Significance: While process control through microbial reduction treatment is predicted to significantly reduce the risk from pistachio consumption, certain pre-processing exceptional situations may contribute to increased risk.

P1-157 A Dynamic Secondary Model to Describe Survival of *Salmonella* in Low-water Activity (a_w) Foods

Steven Duret[1], **SOFIA SANTILLANA FARAKOS**[1], Toluwanimi Ogungbesan[1], Ian Hildebrandt[2] and Susanne Keller[2]
[1]U.S. Food and Drug Administration, College Park, MD, [2]U.S. Food and Drug Administration, Bedford Park, IL

Introduction: Survival of *Salmonella* in low water activity (a_w) foods is, often, characterized by a relatively fast initial decline, followed by long term persistence with slow or no decline over time, which is best described by the Weibull model. Temperature and a_w are the main influencing factors. Storage conditions are rarely constant and there is no available model that can accommodate this.

Purpose: The objective of this study was to develop a general predictive model able to include dynamic conditions for *Salmonella* survival at temperatures below 40°C and a_w levels below 0.7.

Methods: *Salmonella* survival data (86 curves) on tree nuts and spices, temperatures between 4 and 37°C and a_w between 0.11 and 0.68, with measurements of temperature and a_w at every point in the curve, were collected from the literature. A set of differential equations based on the log-linear survival model, including a parameter representing the initial adaptation phase of the cell to the environmental conditions, was fit to the data. A secondary model based on the gamma concept was used to account for the impact of temperature and a_w. Model performance was compared to independent Weibull models using the adjusted R^2_{adj} and the Bayesian Information Criteria (BIC), the latter penalizing a model for its number of parameters.

Results: Statistical analyses show superior performance of the developed model (BIC=1405) as compared to the Weibull model (BIC=1580). The model is more efficient than independent Weibull models, with only a slight decrease in the R^2_{adj} values (0.90 compared to 0.94 for the Weibull models).

Significance: The model simulates survival of *Salmonella* over time, under varying storage conditions. It will increase precision in survival estimates for risk assessments of *Salmonella* in low a_w foods.

Poster

P1-158 Evaluation of Different Animal Feces Levels on Contamination of Leafy Greens Using Sensitivity Analyses of a Mathematical System Model

ABHINAV MISHRA[1], Hao Pang[1], Robert Buchanan[2], Donald W. Schaffner[3] and Abani Pradhan[1]

[1]University of Maryland, College Park, MD, [2]University of Maryland, Department of Nutrition and Food Science and Center for Food Safety and Security Systems, College Park, MD, [3]Rutgers University, Department of Food Science, New Brunswick, NJ

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Introduction: During 1973-2012, *Escherichia coli* O157:H7 was associated with 46 outbreaks in leafy greens, causing 1,584 illnesses and 430 hospitalizations in the US. A large majority of the salad greens consumed in the US are grown within the Salinas region in California. This region has also been associated with the production of leafy greens implicated in several *E. coli* O157:H7.

Purpose: The objective of this study was to develop a system model that simulates the effect of animal feces as a source of contamination in leafy greens, and to conduct a sensitivity analyses to determine the impact of different levels of animal feces on contamination of leafy greens.

Methods: A system model consisting of subsystems and inputs to the system (soil, irrigation, cattle, swine, and rainfall) simulating a hypothetical farm in the Salinas Valley was developed. For sensitivity analyses, the levels of contamination in the baseline model were increased and decreased by up to 100% at intervals of 20% (i.e., ±20%, ±40%, ±60%, ±80%, and ±100%).

Results: At the level of a 100% increase in swine defecation, the mean value of *E. coli* in the harvested crops increased by 1.66- to 4.56-fold compared with the level in the baseline model. When swine defecation was decreased by 100%, the decrease in the mean values was in the range of 1.66- to 11.62-fold. Similarly, when the runoff was increased by 100%, the increase in the mean populations of *E. coli* in the crops was 1.45- to 7.17-fold. The mean population of *E. coli* in the crops was predicted to decrease by 2047- to 8822-fold during April to June and by 1.64- to 6.86-fold during July to November, when the runoff was decreased by 100%.

Significance:

These results could be useful in developing metrics to mitigate the risks of leafy greens associated outbreaks.

P1-159 Reality Check for Handwashing Practices and Guidance for Its Monitoring

NIRAJ SHRESTHA[1], Iryna Sybirtseva[1] and Kayla Simon[2]

[1]Northland Laboratories, Northbrook, IL, [2]Northland Laboratories, Green Bay, WI

Introduction: Lack of proper handwashing techniques by food handlers remains an unresolved issue in the food industry. Handling food products with improperly washed hands poses a food safety hazard that often does not go through comprehensive review and monitoring compared to other likely to occur hazards. Food manufacturers face an uphill task in determining critical control points, establishing critical limits, and monitoring procedures for employee's hands.

Purpose: The purpose of this study is to provide guidance to food manufacturers for development of monitoring program(s) for employee's hands. Specifically, this study will analyze the effectiveness of hand washing frequency and duration, quality of hand washing soaps, and different hand swabbing tools and techniques.

Methods: Twelve subjects were used for this study. They were allocated to (i) handwashing for up to 8 times per day, (ii) handwashing for <10 sec, 30 sec, and >60 sec. The dominant hand of each subject was swabbed from (i) tip of the fingers to bottom of the wrist and (ii) palm only, using cellulose sponge, polyurethane sponge, and letheen broth swabs. The collected samples were enumerated for aerobic plate count (APC), *Enterobacteriaceae* (EB), *Staphylococcus aureus*, yeast, and mold. AOAC petrifilm methods were used for all bacterial enumeration.

Results: In general, no significant difference was observed in bacterial recovery among different swabbing tools. At <10 sec handwashing, APC was 3.31 log for the letheen swabs, 3.63 log for the cellulose sponge, and 4.02 log for the polyurethane sponge. However, the swabbing area played a significant role in APC recovery: the palm had 2 logs less APC than the whole hand with fingers. *Enterobacteriaceae*, *S. aureus*, and yeast were not detected whereas mold was detected on very few subjects.

Significance: This study guides food manufacturers on how to establish effective handwashing practices, develop means of control, and result interpretation with the main goal of creating a 365-degree environmental monitoring program and subsequently safe food production environment.

P1-160 NaCl Upregulates *Icaa* gene of *Staphylococcus aureus*, Increasing Biofilm Formation

SOOMIN LEE[1], Kyoung-Hee Choi[2] and Yohan Yoon[1]

[1]Sookmyung Women's University, Seoul, South Korea, [2]Wonkwang University, Iksan, South Korea

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Introduction: Biofilm produced by *S. aureus* may protect the bacterial cells from antimicrobials and other food-related conditions such as high NaCl concentration.

Purpose: The objective of this study was to elucidate the relation between NaCl and *icaA* gene, and the role of the *icaA* gene in biofilm formation by *Staphylococcus aureus*.

Methods: The *icaA*-deletion mutant of *S. aureus* ATCC13565 was constructed with temperature-sensitive vector, pIMAY. The wild type and Δ*icaA* mutant type of *S. aureus* ATCC13565 were cultured in tryptic soy broth (TSB) plus 0%, 2%, 4%, and 6% NaCl at 35°C for 24 h. A hundred microliter portions of the cultures were plated on tryptic soy agar plus 0%, 2%, 4%, and 6%, followed by incubation at 35°C for 24 h. The colonies on the plates were collected and suspended in phosphate buffer saline to OD_{600} = 0.1, and 20 µl of the suspensions were inoculated in 230 µl TSB plus 0%, 2%, 4%, and 6% NaCl concentration in a 96-well microtiter plate, followed by incubation at 35°C for 9 h. After supernatants of these cultures were discarded, crystal violet was added in each well to quantify biofilm. To visualize the biofilm formation of both *S. aureus* types at 0%, 2%, 4%, and 6% NaCl, confocal laser scanning microscope (CLSM) was used.

Results: Δ*icaA* mutant type of *S. aureus* ATCC13565 was prepared with pIMAY vector. The microtiter plate assay and CLSM showed that the Δ*icaA* mutant produced less biofilm at higher NaCl concentrations compared to that of *S. aureus* ATCC13565 wild type, but biofilm formation by the wild type increased as NaCl concentration increased ($P < 0.05$).

Significance: These results indicate that NaCl causes *icaA* upregulation, increasing biofilm formation of *S. aureus*.

Poster

P1-161 In Vitro and In Vivo Efficacies of Hand Sanitizers against Human Norovirus

BLANCA ESCUDERO-ABARCA, Lee-Ann Jaykus and Rebecca Goulter
North Carolina State University, Raleigh, NC

Introduction: Human norovirus is the leading cause of acute non-bacterial gastroenteritis and foodborn disease. Contaminated hands play a significant role in the spread of the virus, particularly during food handling. Hand sanitizers are purported to interrupt norovirus transmission, but their antiviral efficacy is poorly characterized.

Purpose: This study investigates the efficacy of representative commercial hand sanitizers against human norovirus using in vitro and in vivo assays.

Methods: The current outbreak strain (GII.4 Sydney), obtained as a de-identifed stool specimen was suspended 20% in PBS and used as inoculum. Virucidal suspension assays for in vitro studies (ASTM International standard E1052) and in vivo fingerpad studies using human volunteers (n=10) (ASTM E1838) were done with exposure times of 30 and 60 sec. Virus concentrations, before and after exposure to the sanitizers, including controls, were evaluated by RT-qPCR preceded by RNase treatment.

Results: The benchmark treatment (60% ethanol) produced 0.9±0.09 $\log_{10}$ and 1.7±0.46 $\log_{10}$ reduction in norovirus genome equivalent copies (GEC) after 60 sec by in vitro and in vivo methods, respectively. Comparatively, a commonly used hand sanitizer containing 70% ethanol produced 2.5±0.2 $\log_{10}$ GEC reduction after 60 sec by suspension test, and 2.0±0.4 $\log_{10}$ reduction using the fingerpad method. With a benzalkonium chloride (BAC)-based product, 0.8 ±0.2 and 1.3±0.1. $\log_{10}$GEC reductions were observed after 60 sec for in vitro and in vivo assays, respectively. There were no statistically significant differences (p≥0.05) between benchmark and the two commercial products by in vivo assay, although the ethanol-based product outperformed that containing BAC. Screening of additional hand sanitizers continues.

Significance: The ethanol-based product provided an additional 1.0-1.5 $\log_{10}$ reduction in GII.4 Sydney GEC compared to water alone (0.7±0.2 $\log_{10}$ GEC reduction after 60 sec). The results support the long held belief that hand sanitizers have some antinoroviral efficacy but cannot completely inactivate the virus.

P1-162 Effect of Disinfectants on the Biofilm Formation Capacity of *Listeria monocytogenes* Isolated from Ready-to-Eat (RTE) Meat Products

MAURICIO REDONDO[1] and María Arias-Echandi[2]
[1]University of Costa Rica, San José, Costa Rica, [2]University of Costa Rica, San Pedro, Costa Rica

Introduction: *Listeria monocytogenes* is an opportunistic human pathogen, capable of growing at low temperatures (4°C), that a recognized capacity to form biofilms on food contact surfaces. *Listeria monocytogenes* cells present in biofilms may be more resistant to common disinfectants used at the retail level.

Purpose: The purpose of this study was to evaluate the effect of commonly use disinfectants (chlorine and quaternary ammonium) on the capacity of *L. monocytogenes* cells to establish viable biofilms.

Methods: The biofilm formation capacity of four different *L. monocytogenes* strains was assessed using the plastic microtiter technique. Bacterial cells inoculated into Tryptic Soy Broth (TSB) and peptone water (PW) were exposed to different concentrations of chlorine (200, 300, and 500 ppm) and quaternary ammonium (200, 400, and 600 ppm) for different periods of time (2-6 min). Biofilms were measured by optical density after incubation at 37°C and staining with crystal violet. The reduction of a tetrazolium salt was used to determine the viability of preformed biofilms after exposure to the same antimicrobials. Replicates of the optical density results were analyzed and compared with ANOVA.

Results: Three of the isolates showed higher optical densities (P<0.05) after exposure to high concentrations of chlorine (300 ppm) and quaternary ammonium (600 ppm) for 6 minutes. However, these optical densities were lower (P<0.05) in comparison to the controls (no antimicrobials) and the isolates were classified as moderate biofilm producers. None of the isolates established biofilms in PW. A higher metabolic activity (P<0.05) was observed in the biofilms established by the same isolates; however, some minor activity was observed for other isolates as well.

Significance: These data suggest that disinfection practices in meat stores (type and concentration of disinfectants) may not control the presence of *L. monocytogenes* in these environments.

P1-163 Sanitizing Effectiveness of Electrolyzed Water on *Listeria monocytogenes* and *Listeria innocua* and the Inactivation Mechanism Elucidated by ^{1}h NMR-based Metabolomics

QIN LIU, Ji'en Wu, Zhi Yang Lim and Hongshun Yang
National University of Singapore, Singapore, Singapore

Introduction: *Listeria monocytogenes* is a ubiquitous, intracellular pathogen, causing listeriosis. *Listeria innocua* is, generally, used as a surrogate for *L. monocytogenes* in food microbiological studies. Bactericidal effects of low concentration electrolyzed water (LcEW) on microorganisms are well documented; however, there are few reports on its inactivation mechanism.

Purpose: The purpose of this study was to investigate the lethal and sublethal injury in *L. monocytogenes* and *L. innocua* after LcEW treatments and to characterize the metabolic profile changes and the affected pathways using nuclear magnetic resonance (NMR).

Methods: The efficacy of LcEW in inactivating *L. monocytogenes* and *L. innocua* was evaluated by direct plating on non-selective and selective media. The metabolomic extracts from *L. innocua* were investigated by NMR coupled with multivariate analysis.

Results: The sublethally injured cells of *L. monocytogenes* and *L. innocua* increased from 43.65% to 78.21% and 36.26% to 63.69%, respectively, when the free available chlorine (FAC) of LcEW increased from 0.5 to 8 mg/L. In total, 36 low molecular weight metabolic compounds in *L. innocua* extracts were detected by NMR spectroscopy coupled with multivariate analysis, indicating complex response to oxidative perturbation on the metabolic level. LcEW (4 mg/L FAC) caused significant elevation of 3-hydroxybutyrate, succinate, fumarate, malate, and α-ketoglutarate, but decrease of nucleotides (cytidine, uridine), amino acids (valine, tyrosine, alanine) and ribose-5-phosphate levels. The findings indicated that EW stress severely disturbed the nucleotide and amino acid biosynthesis, energy-associated metabolism, osmotic regulation, and cell wall mucopeptide synthesis.

Significance: This study provided direct evidence that EW at sublethal levels induced oxidative stress in microorganism. The study, also, identified the EW associated metabolic changes in *L. innocua*. These cellular metabolite results might serve as a basis for future mechanistic studies.

P1-164 A Benchtop Drain System to Benchmark Efficacy of Chemicals in Drain Sanitation

Griffin Jadwin[1], **ERIN DALMATA**[2] and Charles Giambrone[3]

[1]Rochester Midland Corporation, Rochester, NY, [2]Rochester Midland Corp, Rochester, NY, [3]Rochester Midland Corporation, New Hope, PA

Introduction: Developed a dynamic flow stainless-steel drain system to generate mixed strain replicate biofilms on surfaces and on polyethylene carriers. These biofilms were then challenged against concentrations of foaming cleaners or sanitizers. Control swabs and carriers were compared against the exposed to determine overall microbial reduction by each individual cleaner from each carrier and surface in the drain.

Purpose: This study is to evaluate foam cleaners and sanitizers on the reduction of pathogens in *E. coli, L. monocytogenes*, and *S. typhimurium* based biofilms in simulated industrial food processing plant drain systems.

Methods: Mixed bacterial cultures were inoculated onto sets of carriers and placed in a sterilized drain trap. A diluted tryptic soy broth solution was delivered by a peristaltic, and air pump between drain traps at a volume of 115 ml every hour. The system was active for 96 ±5 h. The developed mature mixed biofilms (> 6.0 Log cfu per carrier) were determined on each control carrier and the drain trap surface. Test replicate carriers and drain surfaces were then foamed with cleaner for a 5-minute contact time. After exposure, individual carriers were added to neutralizing broth, and drain surface swabs were added to neutralizing broth. Serial dilutions were made of each carrier and swab dilutions, then plated onto tryptic soy agar for comparison against controls to determine log reduction.

Results: The completed cleaners so far show a reduction between a 3-5 log. The highest reduction at 5.20 logs came from the heavy chlorinated alkaline product. The second highest reduction at 4.34 logs was seen from a high concentration peroxyacetic acid, hydrogen peroxide product. The lowest reduction shown was at 3.60 logs from a heavy alkaline, quat, hydrogen peroxide blend product.

Significance: The ability to test foaming drain cleaners and sanitizers will provide food processing sanitarians with a functional database of a variety of cleaning technologies that can be used in effective remediation of a contaminated drain system.

P1-165 Inactivation of *Salmonella enterica* on Food Contact Surfaces during Log, Stationary, and Long-term Survival (LTS) Phases

DORRA DJEBBI-SIMMONS[1] and Wenqing Xu[2]

[1]Louisiana State University, Baton Rouge, LA, [2]Louisiana State University AgCenter, Baton Rouge, LA

Introduction: Contaminated surfaces play a major role in the transmission of bacterial pathogens to food. In the case of *Salmonella*, food contact surfaces can be contaminated through direct contact with raw meat and poultry products. In several environments, bacteria can exist in long-term stationary phase (LTS) occurring after death phase.

Purpose: This study was undertaken to investigate the efficacy of selected commercial disinfectants in the elimination of *Salmonella* from food contact surfaces inoculated with *Salmonella* cells at log, stationary, and LTS phases.

Methods: Laminate, stainless steel, and plastic cutting board surfaces were inoculated with *Salmonella* harvested at log, stationary, and LTS phases, using three different microbial loads (10^6, 10^4, and 10^2 CFU/cm^2). Sodium hypochlorite (0.0095%), acetic acid, and hydrogen peroxide (0.88%) were used for surface disinfection at 0, 2, and 24 h. Residual bacteria were recovered by elution and log reduction of *Salmonella* was calculated. A CRD split plot design was used to analyze results.

Results: All disinfectants reduced surface microbial load, significantly, for all microbial concentrations, for all phases, at all time points, and on all tested surfaces. Hydrogen peroxide had an overall mean log reduction equal to 3.15 $\log_{10}$CFU/cm^2 ± 0.03 compared to 1.86 $\log_{10}$CFU/cm^2 ± 0.03 and 2.97 $\log_{10}$CFU/cm^2 ± 0.03 for sodium hypochlorite and acetic acid, respectively. LTS cells seemed to be more resilient to sodium hypochlorite, with the lowest overall mean log reduction being 1.27 $\log_{10}$CFU/cm^2 ± 0.08 for laminate surface (P<0.001). All main effects for this study (phase, type of surface, time, treatment, and concentrations), as well as interactions, were significant (P<0.004).

Significance: These data suggest that disinfection efficacy of surfaces infected with *Salmonella* depends on multiple factors that must be considered in order to achieve sanitation.

P1-166 Efficacy Evaluation of Commercial Sanitizers on the Decontamination of *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* in Irrigation Well and Pond Water

GANYU GU[1] and Steve Rideout[2]

[1]Virginia Tech, Painter, VA, [2]Virginia Tech - Eastern Shore AREC, Painter, VA

Introduction: Current outbreaks of foodborne illnesses, associated with vegetables and fruits, raised concerns about produce safety. Irrigation water is considered to be one of the main contamination sources of foodborne pathogens on fresh produce. *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* are three common foodborne pathogens that may exist in irrigation water and are reported to be associated with produce contamination.

Purpose: This study was performed to produce science based data, which are needed to generate practical and effective decontamination strategies for irrigation water to mitigate contamination of produce by foodborne pathogens at the production level.

Methods: Well and pond irrigation water were sampled from four vegetable farms on the Eastern Shore of Virginia and inoculated with *S. enterica*, *E. coli* O157:H7, or *L. monocytogenes*. The efficacy of four commercial sanitizers (XY12, CDG Solution 3000™, Sanidate 5.0, and Sanidate 12.0) with different active ingredients was evaluated.

Results: The populations of the three foodborne pathogens were efficiently reduced (>5 log) after 30 min treatment with each tested sanitizers. *Listeria monocytogenes* and *E. coli* O157:H7 were more tolerant to one minute treatments with tested sanitizers compared to *S. enterica*. The pathogen populations were significantly higher in pond water compared to well water, under certain treatments. The depletion of free chlorine and chlorine dioxide with XY12 and CDG3000 (82-96%) were significantly higher than with peracetic acid (3-61%) in Sanidate products. The depletions were not significantly different between pond and well water. Water turbidity was significantly lower in well water compared with pond water (P<0.05). Coliforms and *E. coli* in tested water samples were completely eliminated after 30 min sanitizer treatments. The pH of treated irrigation water was remained in the proper range for plant growth (6-7.5).

Significance: These results provide clues for generating mitigation strategies for foodborne pathogens in irrigation water used for produce production.

P1-167 A Comparison of the Ability of Various Collection Solutions to Neutralize Residual Sanitizers from Environmental Surface Samples

N. Robert Ward and **GEOFF BRIGHT**
World Bioproducts, Bothell, WA

Introduction: Environmental surface samples for microbiological analysis, including the detection of pathogens, are critical in evaluating the effectiveness of cleaning/sanitation programs, as well as maintaining food safety/quality. Residual sanitizers collected with the sample can interfere with growth and recovery of organisms potentially causing false negative test results.

Purpose: This study was conducted to compare the effectiveness of common collection solutions at neutralizing different concentrations of representative sanitizers used in the food processing environment.

Methods: Sterile sponges, hydrated with 10 ml of either Neutralizing Buffer, Letheen Broth, or HiCap(R) were used to collect samples.

Results: Approximately 2.0 logs of *Listeria* were recovered from the control units and samples with the lowest concentration of sanitizers; indicating that all three collection solutions could effectively neutralize approximately 0.2 ml of residual sanitizer. Approximately 2.0 logs were recovered from HiCap and Neutralizing Buffer samples containing the 1.0 ml level of sanitizer for all three sanitizers and from the Letheen Broth samples with hypochlorite sanitizer. Only HiCap demonstrated an ability to neutralize the sanitizers at the 4.0 ml residual sanitizer level. No *Listeria* were recovered from the Letheen Broth samples at the 4.0 ml sanitizer level for any of the sanitizers and 0.8, 0.8, and 0.0 logs of *Listeria* were recovered from the Neutralizing Buffer samples with quat, peroxyacetic acid, and hypochlorite sanitizers, respectively.

Significance: Selection of a collection solution can significantly impact the accuracy of environmental surface test results.

P1-168 Chlorine-based Inactivation of *Escherichia coli* O157:H7: Impact of Residual-free Chlorine Content, Organic Load, Residence Time, and pH

ZI TENG[1], Yaguang Luo[2], Solmaz Alborzi[1], Bin Zhou[2], Boce Zhang[2], Patricia Millner[2] and Qin Wang[1]
[1]University of Maryland, College Park, MD, [2]U.S. Department of Agriculture–ARS, Beltsville, MD

Introduction: During fresh produce washing, the sanitizing efficacy (SE) of chlorine is constantly compromised by organic load (OL), which reportedly contributes to rapid chlorine depletion and unsuccessful maintenance of residual free chlorine (FC). However, it is unclear whether chlorine performs equally well at identical, well-maintained FC residual levels, with and without OL. Knowledge in this regard is important for accurate determination of effective FC levels under high OL.

Purpose: This work was undertaken to develop and use appropriate methodology to evaluate the SE of chlorine under varying OLs, at well-maintained FC levels, relevant to commercial fresh produce washing.

Methods: A sustained chlorine decay approach was employed to evaluate the inactivation of *Escherichia coli* O157:H7 at stabilized FC, chloramine, and chemical oxygen demand (COD) levels. Prepared chlorine-OL solutions were incubated (n=168) at room temperature for four hours to model the effect of residual FC after reacting with OL in a produce wash system. Bacterial survival after 5 and 20 sec exposures to OL-chlorine mixtures was assessed by MPN (n=240).

Results: As OL increased, SE of chlorine decreased. At a 5 s exposure time and pH 6.5, a minimum of 0.5 and 7.5 mg/L FC were needed to achieve a 5 log reduction at 0 and 900 mg/L COD, respectively. The SE decrease was significantly (P<0.05) more pronounced at lower FC, higher COD, higher pH, and shorter exposure time values. The OL-associated interference with FC measurement and disruption of chlorine-bacteria interaction, together with the chlorine demand of concentrated inoculum, collectively resulted in inadequate FC concentration and SE.

Significance: A feasible method for evaluating chlorine-based sanitization was developed and demonstrated the negative impact of OL on SE under well-controlled conditions. To fully account for these chemical interactions in actual commercial-scale produce washing conditions, scale-appropriate evaluations are needed to confirm and incorporate adjustments specific to actual systems.

P1-169 Hydrogen Peroxide-based Disinfectants Inactivate Human Norovirus and Its Surrogate, Tulane Virus

Naim Montazeri, **ERIC MOORMAN**, Jeremy Faircloth, Emma Lepri and Lee-Ann Jaykus
North Carolina State University, Raleigh, NC

Introduction: Human norovirus is a major cause of foodborne illness and effective contact surface decontamination can control its transmission.

Purpose: This study investigated the virucidal efficacy of two commercial disinfectants containing a combination of hydrogen peroxide (5-10%), acids and surfactants, at the recommended use dilution (1:16) against human norovirus GII.4 Sydney and the cultivable surrogate, Tulane virus (TuV).

Methods: The products were tested against the viruses using suspension and carrier (stainless steel coupons) assays according to the American Society for Testing and Materials protocols E1052-11 and E1053-11, respectively, with and without an additional organic load. Log_{10} inactivation was determined based on reduction in genomic copies (GC; RT-qPCR preceded by RNase treatment) for human norovirus, and mammalian cell culture infectivity assay for TuV. The impact of rubbing or removal of virus in conjunction with application of the disinfectants was not evaluated in this study.

Results: In suspension assays testing both products, human norovirus GII.4 Sydney titer was decreased by 4.3 ± 0.3 log_{10} after 20 min, with negligible impact from organic load (P>0.05). The disinfectant efficacy decreased (P<0.05) when tested by surface assay, with an average reduction of 2.6 ± 0.1 log_{10} GC after 20 min in the absence of organic load; after addition of organic load, <1.0 log_{10} inactivation was achieved. Both products fully inactivated infectious TuV (5.5 log_{10} reduction) after 5 min on stainless steel surfaces when no organic load was added; however, after addition of organic load, 30 min was required to achieve a >4 log_{10} reduction.

Significance: Given adequate contact time, both disinfectants were capable of inactivating norovirus, but did not achieve the U.S. EPA standard of ≥4-log_{10} reduction for products claiming virucidal activity. To achieve maximum efficacy for human norovirus decontamination, these disinfectants would best be applied after surface pre-cleaning.

P1-170 Understanding the Efficacy of Sodium Hypochlorite against Norovirus Epidemic Strain GII.4 Sydney

JUSTIN BRADSHAW and Lee-Ann Jaykus
North Carolina State University, Raleigh, NC

Introduction: Surface disinfection is an important tool for controlling norovirus outbreaks. However, current CDC guidelines for surface disinfection are often too high for practical use in commercial settings. Better resolution of surface disinfection efficiency of the common disinfectant, sodium hypochlorite, against norovirus can enhance outbreak control in instances when CDC guidelines are difficult to implement.

Purpose: This study determined the concentration of sodium hypochlorite and contact times necessary to ensure significant reduction of representative human norovirus GII.4 Sydney from stainless steel surfaces.

Methods: Clarified 20% suspensions of human feces confirmed positive for GII.4 Sydney were used with or without the addition of a tripartite soil load. Aliquots were dried on stainless steel coupons and exposed to sodium hypochlorite (concentrations from 100 PPM – 5,000 PPM) for contact times ranging from 1-30 min. Following neutralization and elution, the suspension was subjected to RNAse pre-treatment and RT-qPCR. Log_{10} reduction was calculated based on genomic copies. Triplicate samples were tested per data point.

Results: For treatment in the absence of soil load, a 750 PPM sodium hypochlorite solution produced a 5.3 ± 0.5 log_{10} genome copy number reduction after a 1 min contact time and a solution as low as 400 PPM showed 5.1 ± 0.5 log_{10} reduction after 5 min. Addition of a supplemental soil load (5%) to clarified virus significantly reduced disinfectant activity, such that a 5000 PPM sodium hypochlorite solution resulted in only a 3.4 ± 0.2 log_{10} reduction in genome copy number after a 1 min exposure.

Significance: For clean surfaces, a 400 PPM sodium hypochlorite solution is the minimum concentration necessary to reliably cause a 4 log_{10} reduction in genome copy number with a contact time less than 5 min. The presence of excess soil almost completely abolished disinfectant efficacy. It may be possible to use lower concentrations of free chlorine, if thorough surface cleaning is done prior to disinfection.

P1-171 Efficacy and Stability of Disinfectant Solutions Applied to Papaya (*Carica papaya*) at Packing Facilities in Mexico

JORGE ADRIÁN MUNIZ FLORES[1], Jennifer A. Chase[2], Edward R. Atwill[2], Ma. Ofelia Rodríguez-García[1] and Elisa Cabrera-Diaz[1]

[1]Universidad de Guadalajara, Guadalajara, Mexico, [2]University of California-Davis, Davis, CA

Introduction: Papaya exported from Mexico has been associated with *Salmonella* outbreaks and import alerts. Postharvest, papayas are immersed in subsequent tanks to wash and disinfect. The first tank (A) contains chlorine and a stabilizer and the second tank (B) contains peroxyacetic acid and thiabendazole. The efficacy of these solutions to control *Salmonella* has not been evaluated.

Purpose: To evaluate the efficacy and stability of disinfectant solutions used in papaya packing facilities in Mexico.

Methods: Solutions were prepared according to industry standards and held at room temperature: solution-A contained 1.5ml/L of sodium hypochlorite (13% v/v) and 0.5ml/L of AquaRite stabilizer™; solution-B had 1.5ml/L of SaniDate5.0™ and 1.5g/L of Tecto60™. Free chlorine, ORP, temperature and pH were measured after 0, 2, 4 and 6h. At each time interval, 5ml of each solution were inoculated with a cocktail of six *Salmonella* serotypes including, Bredeney, Muenchen, C1, Oranienburg, Minnesota, and Typhimurium (6.3±0.2-log CFU/ml). After 30s, 5ml of double strength D/E neutralizing broth was added. *Salmonella* was enumerated on tryptic soy agar. Experiments were conducted in triplicate (n=72). Physicochemical parameters and *Salmonella* reductions were analyzed with ANOVA and LSD test (α=0.05).

Results: Solutions A and B had a mean ORP of 213±1.3 and 180±1.4mV, and pH of 3.3±0.3 and 3.8±0.2, respectively; these values were not significantly different (P>0.05) after 6h. Free chlorine concentration was 231±56mg/L with a slight reduction after 6h (P>0.05). Overall mean *Salmonella* reductions were 2.5±1.0 and 3.6±1.4 log CFU/ml in A and B, respectively; lower reductions were observed after 6h (P<0.05).

Significance: Under controlled conditions, the disinfectant solutions were stable after 6h, however, exhibited limited efficacy to reduce *Salmonella* after 30s of contact time. Additional field studies will evaluate the efficacy of disinfection solutions, to determine if an inhibitory effect occurs when soil or debris are introduced by harvested papaya.

P1-172 Efficacy of Sanitizers in Inactivating Fecal Coliforms in Cell Cultures and on Coupons Made from Blueberry Contact Surface Materials

HIMABINDU GAZULA[1] and Jinru Chen[2]

[1]University of Georgia, Griffin, GA, [2]University of Georgia, Department of Food Science and Technology, Griffin, GA

Introduction: Bacteria attached to blueberry contact surfaces can cause potential cross-contamination, which can lead to fruit spoilage and possible food safety concerns.

Purpose: This study evaluated the ability of three sanitizers in inactivating fecal coliforms in cell cultures and on coupons made from blueberry contact surface materials.

Methods: Five bi-strain mixtures of fecal coliforms (10^5 CFU/ml) were exposed to 5 ppm active chlorine dioxide (ClO_2), 200 ppm quaternary ammonium (QAC) or 3 ppm ozonated water (OW) for 1 minute. In a separate study, 100 µl of each cell suspension was inoculated on coupons (2×5 cm^2) made of high-density polyethylene, stainless steel, rubber, polyvinyl chloride, polypropylene and polyurethane. The surfaces were air dried for 4 h at 10°C before being treated with aforementioned sanitizers. Surviving cells in both assays were enumerated on LB and MAC.

Results: In the cell culture assays, ClO_2 caused an average of 3.01 log reduction whereas QAC and OW both resulted in a 4.97 log reduction on MAC. On LB, a 2.77, 5.15 and 5.12 log reduction was obtained by treatment with ClO2, QAC and OW, respectively. On average, only 0.64, 1.51 and 1.38 log reductions were observed on LB when the contact surfaces were treated with ClO_2, QAC or OW. On MAC, treatment with ClO_2, QAC and OW caused 1.13, 2.04 and 1.94 log reductions. Overall, no significant difference was observed in the performance of QAC and OW. However, their efficacies were significantly higher than the efficacy of ClO_2. Polypropylene and polyvinylchloride had the highest, whereas high-density polyethylene had the lowest bacterial counts after sanitation.

Significance: QAC and OW are more effective than ClO_2 for fecal coliform inactivation. The type of bacterial contact surface had a significant influence on the efficacy of sanitizers. Dried bacterial cells on their contact surface are much more difficult to inactivate than those in liquid cultures.

P1-173 The Combined Effects of Physical Removal, Proper Cleaner Selection and Sanitizer on the Reduction of a *Pseudomonas aeruginosa* Biofilm

Igor Ignatovich, Joshua Luedtke, Oriana Leishman and **TERESA PODTBURG**

Ecolab Inc., St. Paul, MN

Introduction: Many studies on biofilm treatments have investigated the efficacy of sanitizers alone. In order to fully understand the dynamics involved in the reduction of biofilm from surfaces, we examined the effects of physical removal, cleaner selection and use of a peracid based sanitizer alone and in combination.

Purpose: This study was performed to examine the combined effects of physical removal, cleaner selection and sanitizers on the reduction of *a Pseudomonas* biofilm.

Methods: A *Pseudomonas aeruginosa* (ATCC 15442) biofilm was grown in a CDC Biofilm reactor on 304 stainless steel carriers following methodology adapted from the ASTM E2562 standard. Biofilm coated carriers were exposed to various treatment combinations consisting of physical removal (PR), a non-chlorinated alkaline cleaner (NC-AC), a chlorinated alkaline cleaner (C-AC) and a peracid sanitizer. Following exposure, carriers were neutral-

Poster

ized, biofilm disaggregated via a series of vortex/sonication steps and survivors enumerated by serial dilution and plating onto Tryptic Soy Agar + 5% Sheep's Blood. Log survivors were calculated for comparison of each treatment combination.

Results: Data demonstrates that the additive effects of PR, cleaners and sanitizers are significantly different ($P<0.05$) then each of the treatments alone. PR provided < 0.5 log reduction, the cleaners + PR from 0.5 to 4.0 log reduction and the sanitizer alone a 1.0 log reduction. From least effective to most effective: PR < NC-AC +PR < sanitizer < sanitizer + PR < NC-AC + PR + sanitizer < C-AC + PR < C-AC + PR+ sanitizer.

Significance: This study verifies that the performance of a full cleaning process with appropriate cleaner selection is essential for effective management of biofilms.

P1-174 Selection of Alternative Indicators for Monitoring the Washing Effect of Salted Cabbages

EUNSOM CHOE, Sunghyuk Bang, Seulki Byeon, Gyiae Yun and Ki-Hwan Park

Chung-Ang University, Anseong, South Korea

Introduction: The washing process is an important step to reduce the level of microorganisms in fresh fruits and vegetables. The real-time effectiveness of washing is hard to be evaluated since it is impossible to measure the microorganism levels on vegetables during continuous washing step.

Purpose: The purpose of this study was to select alternative indicators equivalent to assure the level of microorganisms in salted vegetables in washing processes.

Methods: Throughout the four washing steps, salted cabbages and washed waters were collected, and turbidity and salinity in washed water were measured at predetermined time intervals. The monitoring was repeated for nine months. The level of total aerobic counts and coliforms in cabbages and waters were analyzed. The correlation between the number of total aerobic counts and turbidity or salinity were evaluated.

Results: The level of total aerobic counts in cabbages and waters, turbidity and salinity were decreased with washing steps, while increased with processing time. The range of turbidity was 0-50 NTU while 0-5% of salinity. About 0.8 log CFU/g of total aerobic counts in cabbages, and 0.8-1.2 log CFU/g, 0.8-1.4 log NTU of turbidity and 2% of salinity in washed water were increased with processing time, which means that enough water should be supplied continuously to maintain the level of microorganisms during washing steps. The log CFU/g of total aerobic counts was well correlated with the log scale of turbidity ($R^2 = 0.942$) and salinity ($R^2 = 0.804$), and the 10 NTU of turbidity and 1% of salinity indicated about 5.0 log CFU/g of total aerobic counts in washed water.

Significance: These results suggest that the real-time monitoring of turbidity system combined with control of water supply would be applied to maintain the washing effect of fresh vegetables.

P1-175 Evaluating Current Industry Dry Cleaning Practice Using Vacuum with Regard to Food Allergens on Processing Surfaces

CHRISTOPHER WELLS[1] and Sanghyup Jeong[2]

[1]Self, St. Johns, MI, [2]Michigan State University, East Lansing, MI

Introduction: Allergenic cross-contamination is a growing concern for the food industry, which has been reflected in increasing number of incidences of undeclared allergen contamination according to USDA. Especially, the cleaning methods used in low moisture environments where water cannot be used is an area of concern. Therefore, evaluating current industry practices is necessary to determine if new methods are required or current methods are satisfactory.

Purpose: The purpose of this study was to determine the effectiveness of the vacuum dry cleaning method used in the food industry.

Methods: Stainless steel coupons were electrostatically coated with soy protein isolate powder as an allergenic material. The coupons were then subjected to vacuum cleaning at about 3 mm above the surface. After 10 seconds of vacuuming followed by a brushing, second vacuuming was applied for 10 seconds. Then, the coupons were tested for the presence of the soy allergen using Neogen 3D Reveal test kits. For cleaning efficacy, another set of coupons was vacuum treated, after which the coupons were submerged and agitated in 15 mL of deionized water in a plastic bag. The total dissolved solids (TDS) and conductivity were measured for the wash water using a water quality meter.

Results: The allergen tests showed 50% negative and 50% positive for soy ($n=6$), which indicates the uncertainty of the vacuum cleaning practice for allergen removal. The mean TDS, 4.72 ± 1.57 ppm and the conductivity, 3.7 ± 0.5 micro S/cm, supports the mixed allergen test results. The results of the vacuum cleaning test provided further evidence that visual cleanness poses the risk of allergen cross-contamination.

Significance: Although the current industry vacuum cleaning practice is considered as an effective and practical dry cleaning method, it needs to be further validated and improved to ensure allergen safe food products.

P1-176 Withdrawn

P1-177 Withdrawn

P1-178 Cross-contamination of Human Pathogens from Pressed Paper and Bamboo Cutting Boards to Tomato and Kale

HOLLY PADEN, Kevin Mo, Kristin Motil and Sanja Ilic

Ohio State University, Columbus, OH

Introduction: Contamination of fresh produce with human pathogens remains an important public health threat. Cross-contamination from unclean cutting boards to ready-to-eat foods is a documented route of pathogen transmission. Cutting boards made with materials produced from recycled and sustainable sources, such as pressed paper and bamboo, are becoming increasingly popular. These cutting boards are used daily in households and restaurants to prepare fresh produce for service to millions of people. However, there is limited research available on transmission of human pathogens from these surfaces.

Purpose: This study was conducted to quantify the transmission of *Listeria monocytogenes* and *Salmonella* spp. from contaminated cutting boards to fresh tomato and kale.

Methods: Cocktail inocula of *L. monocytogenes* and *Salmonella* spp. were used to spot inoculate stainless steel, bamboo, and pressed paper surfaces. Kale leaves and whole grape tomatoes were pressed to surface coupons, with an equal amount of force (approximately 320g), for three seconds. The samples were homogenized and serial dilutions were made for enumeration, after 24 hours of incubation at 37ºC. The experiments were repeated eight times. The concentrations were calculated and the collected data were analyzed using SPSS.

Results: The transfer of pathogens to kale [3.83±0.42 log CFU/mL] was consistently higher than to tomatoes [3.63±0.51 log CFU/mL] [*P*<0.05]. Bacteria transferred at a similar rate from all types of surfaces (cut bamboo, bamboo, stainless steel, cut paper, and paper) [*P*=0.310] under the studied conditions (wet surfaces). The transfer of *Salmonella* spp. [3.74±0.54 log CFU/mL] was significantly higher than that of *L. monocytogenes* [3.71±0.39 log CFU/mL] [*P*=0.01].

Significance: The data from these experiments highlight the importance of maintaining sanitary kitchen surfaces. For food safety, clean cutting boards must be used to cut and prepare fresh produce, as pathogens on the surface of cutting boards can be readily transmitted.

P1-179 Histamine-related Hygienic Quality and Adulteration with Pork or Poultry in Commercial Dried Fish Floss Products

YUNG-HSIANG TSAI[1], Yi-Chen Lee[1], Hsien-Feng Kung[2] and Pei-Hsiang Lee[1]

[1]National Kaohsiung Marine University, Kaohsiung City, Taiwan, [2]Tajen University, Pingtung City, Taiwan

Introduction: Histamine is the causative agent of scombroid poisoning, a foodborne chemical hazard that is usually ingested with tuna, bonito, marlin, mahi-mahi and mackerel, which contain high levels of free histidine in their muscle. In Taiwan, dried fish floss product is a dried fish meat (including tuna, marlin, and salmon) with a light, fluffy texture similar to coarse cotton.

Purpose: The purpose of this study was to evaluate the histamine-related hygienic quality in ten dried fish floss samples manufactured from Hazard Analysis and Critical Control Points (HACCP) seafood processing factories and fifty-nine samples manufactured from non-HACCP factories.

Methods: The levels of total volatile basic nitrogen (TVBN), aerobic plate count (APC), Coliform count, histamine concentration, and authentication of meat species in samples from HACCP and non-HACCP factories were determined.

Results: None of these samples contained Coliforms or *E. coli*. The levels of TVBN and APC, in all samples, were less than Chinese Agricultural Standard limits of 25 mg/100g and 5.0 log CFU/g, respectively. The histamine content in all samples was less than 0.22 mg/100g, which was below the 5.0 mg/100g allowable limit suggested by the U.S. Food and Drug Administration. Assays of multiplex polymerase chain reaction (PCR) revealed adulteration rates of 34.8% (24/69) and 2.90% (2/69) for poultry and pork, respectively. All adulterated samples were collected from non-HACCP factories.

Significance: The data showed that histamine-related hygienic quality and adulteration with pork or poultry meats in HACCP factory samples were better than those of non-HACCP factory samples.

P1-180 Food Deserts and Food Safety: An Examination of the Microbial Profile of Leafy Greens from the Houston Area High-income and Low-income Grocery Stores

KRISTINA INFANTE[1] and Sujata A. Sirsat[2]

[1]University of Houston, Conrad N. Hilton College of Hotel and Restaurant Management, Houston, TX, [2]University of Houston, Houston, TX

✦ ***Undergraduate Student Award Competitor***

Introduction: Food deserts are a prevailing issue in the greater Houston area and the little fresh produce available to low-income neighborhoods could be exposed to poor handling procedures and improper holding temperatures.

Purpose: The purpose of this study was to identify pathogenic bacteria, APCs, coliforms, *Escherichia coli*, and yeasts/molds on romaine lettuce samples purchased from Houston area grocery stores (low- and high-income neighborhoods).

Methods: Romaine lettuce was purchased from five high-income (HI) and five low-income (LI) grocery stores, based on household median income. Standard methods of detection, as per the Bacteriological Analytical Manual-Food and Drug Administration, were used with some modifications. The lettuce was aseptically weighed and added to a sterile stomacher bag with 45mL peptone buffer, stomached for 120s, serially diluted, and plated on APC and coliform/*E.coli* petrifilms. Samples were also enriched for selective enumeration using the following media: PALCAM (selective for *Listeria* spp.), EC Medium with novobiocin (*E. coli* O157:H7), and Tetrathionate broth (*Salmonella* spp.).

Results: The APC results demonstrated a statistically significant difference (*P*<0.05) between lettuce obtained from HI (2.9 Log CFU/g) and from LI (4.7 Log CFU/g) grocery stores, respectively. In addition, the coliform and *E. coli* results demonstrated a statistically significant difference (*P*<0.05) between HI (2.9 Log CFU/g) and LI (4.9 Log CFU/g) sampling locations. The PALCAM enriched LI sample exhibited growth. Subsequent plating on Sheep Blood Agar displayed β-hemolysis, suggesting the presence of *Listeria monocytogenes.*

Significance: The results of this study highlighted the inconsistencies in the handling/holding procedures of Houston grocery stores. There is an increasing need for accessibility of food safety related educational materials for grocery store employees/managers that serve low-income neighborhoods.

P1-181 Antibacterial Efficacy of Eugenol against *Escherichia coli* O157:H7 and *Salmonella enterica* in Unpasteurized Apple Juice Produced in Juice Bars and Held at 4°C

ARMITRA JACKSON-DAVIS[1], Aubrey Mendonca[2], Floyd Woods[3] and Salam Khan[4]

[1]Alabama A&M University, Madison, AL, [2]Iowa State University, Ames, IA, [3]Auburn University, Auburn, AL, [4]Alabama A&M University, Normal, AL

Introduction: Growing consumer demand for minimally processed foods devoid of synthetic preservatives has fueled increased consumption of unpasteurized juices produced in juice bars. Juice bars do not apply a kill step for pathogens and unpasteurized juices have been implicated in several foodborn disease outbreaks.

Purpose: The purpose of this study was to examine the antibacterial effect of eugenol, a component of clove oil, against *Escherichia coli* O157:H7 and *Salmonella enterica* in refrigerated, unpasteurized apple juice.

Methods: Unpasteurized apple juice with added eugenol (0.25, 0.5, and 1.0 µl/ml), was inoculated with a 5-strain mixture of *E. coli* O157:H7 or *S. enterica* to obtain an initial viable count of 7.0 log CFU/ml. Juice without added eugenol served as control. Viability of pathogens in the juices (4°C), at set times during 24 hours, was evaluated by plating diluted (10-fold) juice samples on sorbitol MacConkey agar (*E. coli* O157:H7) and xylose lysine tergitol 4 agar (*S. enterica*) and counting bacterial colonies on agar media after incubation (35°C, 48h).

Results: In juice with or without added eugenol, no growth of the pathogens occurred at 4°C. Viable pathogens in control juice decreased by approximately 0.7 to 1.0 log CFU/ml at 24 h. *Salmonella enterica* was more sensitive to eugenol (1.0 µl/ml) compared to *E. coli* O157:H7. In juice containing eugenol at 0.25 µl/ml, viable *E. coli* O157:H7 and *Salmonella* decreased by approximately 3.62 and 4.24 log, respectively, after 4 h. Eugenol (0.5 or 1.0 µl/ml) inactivated both pathogens by more than 5.0 log after 4 h and none of the pathogens was detected after 24 h in juice with the same eugenol concentrations (*P* <0.05).

Significance: Eugenol has good potential for use as a natural antimicrobial for inactivating bacterial pathogens in refrigerated unpasteurized apple juice.

P1-182 Effectiveness of Citric/Lactic Acid Solution Alone or Combined with Added Linoleic Acid for Inhibiting *Salmonella enterica* and *Escherichia coli* O157:H7 on Chicken Skin

Loutrina Staley[1], **ARMITRA JACKSON-DAVIS**[2], Aubrey Mendonca[3], Leopold Nyochembeng[1] and Ernst Cebert[1]
[1]Alabama A&M University, Normal, AL, [2]Alabama A&M University, Madison, AL, [3]Iowa State University, Ames, IA

Introduction: *Salmonella* spp. and *Escherichia coli* continue to be pathogens of concern in the poultry industry. Current interventions are limited in their effectiveness to destroy pathogenic bacteria. The use of plant fatty acids and organic acids may show some promise in fulfilling the demand for natural antimicrobials.

Purpose: The objective of this study was to determine the efficacy of plant fatty acids in combination with organic acids on *Salmonella* spp. and *E. coli* O157:H7 on chicken thigh skin.

Methods: Chicken thigh skin was inoculated by dipping into 50 ml of 8 log CFU/ml of *Salmonella* spp. and *E.coli* O157:H7 and then allowed to attach. After attachment, the following treatments were applied by dipping the inoculated chicken thigh skin in the following treatment: (1) 18.75% Citric/lactic acid (CLA), (2) 18.75% CLA + 0.675% linoleic acid, (3)18.75% CLA + 1.35% linoleic acid, (4) Distilled H_2O, and (5) 20 ppm chlorinated water. After treatment, samples were stored over for eight-days. On specified days, the number of surviving cells was determined by spread plating (on SMAC for *E. coli* and XLD for *Salmonella* spp.) and incubating for 24 hours at 37°C.

Results: Results show that CLA and CLA+linoleic acid were significantly ($P < 0.01$) effective in reducing *Salmonella* spp. and *E. coli* on chicken thigh skin without negatively affecting the color of the chicken thigh skin. Treatments for four minutes successfully reduced the *Salmonella* spp. by five log CFU/g and the *E. coli* O157:H7 by six log CFU/g. Treatments 3 and 4 reduced both pathogens by eight log on the final sampling day. Control treatments only reduced both pathogens by two log CFU/g.

Significance: CLA solution alone or with added linoleic acid can serve as natural antimicrobials in controlling the survival of *Salmonella* spp. and *E. coli* on stored chicken skin.

P1-183 Molecular Characterization of Antimicrobial-resistant Non-typhoidal *Salmonella enterica* Serovars from Imported Food Products

ASHRAF KHAN and Dongryeoul Bae
U.S. Food and Drug Administration/NCTR, Jefferson, AR

Introduction: Unlike mild gastroenteritis by non-typhoidal *Salmonella* (NTS) infection, severe systemic infections, including bacteremia or meningitis, can occur in immunocompromised persons. Fluoroquinolones and β-lactams are widely used to treat the life-threatening systemic infections. However, the emergence of multidrug resistant strains is a global health concern.

Purpose: The objective of this study was to determine antimicrobial resistance in 115 NTS isolates from food products imported into the US during last four years.

Methods: One hundred fifteen NTS were isolated from a variety of imported food products (n= 3,840). Disk diffusion agar and microdilution assays were performed for antimicrobial susceptibility testing using 10 antimicrobials. A combination of PCR, DNA sequencing, and plasmid analyses were performed to characterize antimicrobial resistance determinants.

Results: Twenty-four out of 115 *Salmonella enterica* isolates were resistant to various antimicrobial classes including beta-lactam, aminoglycoside, phenicol, sulfonamide, glycopeptide, trimethoprim, and/or fluoroquinolone antimicrobials. Twelve of the isolates were multi-drug resistant strains. Antimicrobial resistance determinants, including bla_{TEM-1}, $bla_{CTX-M-9}$, bla_{OXA-1}, *tetA*, *tetB*, *tetD*, *dfrA1*, *dfrV*, *dhfrI*, *dhfrXII*, *drf17*, *aadA1*, *aadA2*, *aadA5*, *orfC*, *qnrS*, and mutations of *gyrA* and *parC*, were detected in one or more antimicrobial resistant NTS isolates. Plasmid profiles showed that several antimicrobial resistant strains harbored plasmids having incompatibility groups IncFIB, IncHI1, IncI1, IncN, IncW, and IncX.

Significance: Our study indicates that imported foods contaminated with multidrug resistant NTS may contribute to the spread of antimicrobial resistance genes and potentially compromise the therapeutic activity of antimicrobials. In addition, the data generated from the integrated research approaches reported in this study could be useful for investigations of foodborne illness outbreaks linked to domestic and international food facilities.

P1-184 Antimicrobial Resistance of *Salmonella* spp. Isolated from Retail Beef and Beef Cattle during Harvesting in Honduras

DIEGO CASAS, Brenda Inestroza, Alejandra Ramirez, Mindy Brashears, Mark Miller and Alejandro Echeverry
Texas Tech University, Lubbock, TX

Introduction: *Salmonella* is a leading cause of foodborne illness worldwide. Antimicrobial and multidrug resistant *Salmonella* may be present throughout the food chain and can be a possible source of infection via consumption of contaminated products.

Purpose: This study compared the antimicrobial resistance of *Salmonella* isolated from retail beef and beef cattle, at harvest, in Honduras.

Methods: Antimicrobial resistance of *Salmonella* isolates from beef cattle (n=47) and retail beef (n=30) in Honduras was determined by microbroth dilution. The minimum inhibitory concentrations (MIC) of nine antimicrobial classes were estimated. Values of MIC were obtained using the Sensititre® OptiRead™ system and Sensititre® software SWIN®(V3.3). Results were categorized as resistant, intermediate, or susceptible according to the manufacturer's instructions.

Results: A total of 86.7% (26/30) of isolates from retail beef were deemed resistant, with 3.3% (1/30) intermediate, and 10.0% (3/30) susceptible to at least one antimicrobial class. Moreover, isolates from beef cattle were 72.3% (34/47) resistant with 8.5% (4/47) intermediate and 19.2% (9/47) susceptible to at least one antimicrobial. Among the resistant isolates from retail beef and beef cattle, 65.4% (17/26) and 41.2% (14/34), respectively, were multidrug resistant. In both cases, resistance to β-lactams was similar (55.6% [15/27] in retail vs 47.4% [18/38]) during harvest. The penicillin resistance rate was significantly different between retail and harvest isolates (59.3% [16/27] vs 23.7 % [9/38]). Furthermore, fluoroquinolone resistance was significantly different with 29.6% (8/27) in retail and 68.4% (26/38) during harvest.

Significance: Contamination of beef products with antimicrobial and multidrug resistant *Salmonella* may result in foodborne illnesses that are difficult to treat. There is a variation of antimicrobial resistant *Salmonella* throughout the meat chain, where certain resistances may be mitigated while others may be enhanced. Further investigations are required to understand their origin and implement food protection strategies.

P1-185 Prevalence of Resistant *Salmonella* spp. Isolated from Pasteurized Cow Milk and Its Related Samples in the Tamale Metropolis of Ghana

ADZITEY FREDERICK, Patricia Asiamah and Courage Kosi Setsoafia Saba
University for Development Studies, Tamale, Ghana

Introduction: Contamination of milk and milk products by *Salmonella* spp. and their resistance to antibiotics is a threat to public health.
Purpose: This study was conducted to determine the prevalence of resistance *Salmonella spp.* isolated from milk and milk products in Tamale, Ghana.
Methods: Three hundred milk and milk-related samples were examined. Isolation of *Salmonella* species was done according to the U.S.-FDA Bacteriological Analytical Manual. Antibiotic susceptibility tests were performed by disc diffusion and the results were interpreted using the CLSI guidelines. Prevalence data was analyzed using SPSS Version 17.
Results: Of the 300 samples examined, 7.3% (22) were positive for *Salmonella* spp. Raw wagashie was most contaminated (24.0%; 12/50), followed by fried wagashie (8.0%; 4/50), pasteurized milk (6.0%; 3/50), left hand swab of milk sellers (4.0%; 2/50) and right-hand swab of milk sellers (2.0%; 1/50). *Salmonella* spp. were not isolated from brukina (0.0%; 0/50). There were no significant differences ($P > 0.05$) among fried wagashie, pasteurized milk, hand swab of milk sellers, or brukina samples. The prevalence of *Salmonella* in raw wagashie was significantly higher ($P < 0.05$) than the other samples examined. From the 22 *Salmonella* spp. isolates examined against 8 different antibiotics, a high percentage (86.0%) was resistant to erythromycin. Resistance to gentamycin (14.0%) and tetracycline (14.0%) were relatively low. *Salmonella* spp. isolates were highly susceptible to ciprofloxacin (100.0%), chloramphenicol (91.0%), ceftriaxone (91.0%), sulphamethoxazole/trimethoprim (91.0%), tetracycline (86.0%), and ampicillin (86.0%). Of the 22 *Salmonella* spp. isolates, 14 were resistant to only one antibiotic, 4 were resistant to two antibiotics, and 2 were resistant to three antibiotics. Two *Salmonella* spp. isolates were not resistant to any of the antibiotics tested.
Significance: This study create the awareness that some milk, milk products, and hands of milk sellers in the Tamale Metropolis are contaminated with *Salmonella* spp., which are resistant to some antibiotics. Therefore, consumers of milk in this metropolis are at risk of *Salmonella* spp. infection.

P1-186 Synergistic Effect of X-Ray Irradiation and Sodium Hypochlorite or Chlorine Dioxide against *Salmonella* Typhimurium Biofilm on the Quail Eggshells

SOO-JIN JUNG, Shin Young Park, Hye-Ran Cho, Do Hyoung Kim and Sang-Do Ha
Advanced Food Safety Research Group, Brain Korea 21 Plus, Chung-Ang University, Ansung, South Korea

Introduction: *Salmonella* Typhimurium is one of the common foodborne pathogens in eggs, owing to its ability to contaminate egg contents through the egg shell. *Salmonella* Typhimurium can form biofilms on food contact surfaces and egg shells. Biofilms are a great concern in food safety because they are resistant to chemical agents such as sanitizers, cleaning agents, and disinfectants.
Purpose: The present study aimed to evaluate the synergistic effect of combined treatments of x-ray and sodium hypochlorite (NaOCl) or chlorine dioxide (ClO_2).
Methods: Combination treatments were conducted using x-ray irradiation (0.5, 1.0, 1.5, and 2.0 kGy) and NaOCl (50, 100, 150, 200, and 300 ppm) or ClO_2 (10, 20, 30, and 50 ppm) to reduce *S.*Typhimurium ATCC 14028 biofilms on quail egg shells. Additionally, the color change of quail egg shells was measured by Hunter color (L, a, b, and ΔE). Also, we tested puncture force to evaluate thickness of egg shell after combined treatments.
Results: The highest reduction values for the biofilms were observed as 4.61 and 4.67 log CFU/egg after x-ray/NaOCl (2.0 kGy/300 ppm) and x-ray/ClO_2 (2.0 kGy/50 ppm), respectively. The synergistic reduction values of biofilms after combined treatments were 1.47 log CFU/egg (total 4.28 log reduction) by 2.0 kGy x-ray/50 ppm NaOCl, and 1.07 log CFU/egg (total 4.67 log reduction) by 2.0 kGy x-ray/20 ppm ClO_2. The Hunter color (L, a, and b) on quail egg shell was not significantly ($P > 0.05$) changed by any combined treatment. The ΔE observed, after combined treatments, was 0.55-1.43 units . The puncture force (g) of quail egg shells was not significantly ($P > 0.05$) changed by any of the combined treatments.
Significance: The 2.0 kGy x-ray/50 ppm NaOCl and 2.0 kGy x-ray/20 ppm ClO_2 combination treatments could be considered optimal for eliminating *S.* Typhimurium biofilms on egg shells, without any color and thickness changes.

P1-187 Effectiveness of Yogurt and Kefir in Reducing *Salmonella* spp. Numbers on Chicken Skins

HUSNU SAHAN GURAN
Dicle University, Department of Food Hygiene and Technology, Diyarbakir, Turkey

Introduction: Yogurt and kefir are the most known and consumed fermented milk products. These products contain many beneficial microorganisms, such as lactic acid bacteria. These good microorganisms have been used to fight against common foodborne pathogens including *Salmonella* spp.
Purpose: This study was carried out to determine the effect of yogurt and kefir on the fate of *Salmonella* on raw chicken skins.
Methods: Raw chicken skin pieces (20 cm^2) were artificially contaminated with *Salmonella* and divided into four groups. The groups included a control group (C): non treated with yogurt or kefir; Group I: treated with yogurt; Group II: treated with kefir; and Group III: treated with yogurt plus kefir. All the groups were stored at 4±1°C in a cooled incubator for 6 days. Analyses for the numbers of *Salmonella* in the samples were performed at 0, 3, and 6 days of storage. The *Salmonella* spp. were enumerated on XLT4 agar and the colonies were counted after 18-24 h of incubation at 37°C.
Results: No significant differences were found between groups during the storage period ($P > 0.05$). At the end of the storage (6^{th} day), Group I and Group II showed reduced *Salmonella* populations by 0.56 and 0.53 log cfu/cm^2, respectively, while the reduction for Group III was 1.08 log cfu/cm^2.
Significance: The results of this study indicate that the combined use of yogurt plus kefir is more effective than yogurt or kefir alone in reducing *Salmonella* numbers on chicken skins.

P1-188 Use of LED Ultraviolet (UV) Light for the Reduction of *Salmonella* sp. on Surface of Chicken and Food Contact Surfaces

M. ALEXANDRA CALLE, Ilan Arvelo, Brayan Montoya, Jon Thompson and Mindy Brashears
Texas Tech University, Lubbock, TX

Introduction: The use of ultraviolet light has been proven to be effective for microbial inactivation. Ultraviolet light-emitting diodes (UV LED) have been broadly used for water disinfection but to date other applications are scarce. Due to public concern about the use of chemicals or thermal treatments modifying food characteristics, UV LEDs could be an alternative for microbial control.
Purpose: To investigate the use of an UVC LED (wavelength 250-280 nm, 20mW) to reduce *Salmonella* sp. on various surfaces.

Methods: Boneless skinless chicken breasts (CB) (2x2 cm), as well as flat stainless steel (SS) and teflon (TF) coupons (2x2 cm) were surface inoculated with a five-strain *Salmonella* cocktail at a target concentration of 6.5 Log_{10}(CFU/cm^2). Samples were treated with UVC LED light for up to 15 min. SS and CB (45 and 51 samples, respectively) were treated with varying average irradiance between 0–2 mW/cm^2. A second study was conducted with CB and TF (54 samples each) using 0–4 mW/cm^2 irradiance. After treatments, viable colonies were enumerated by plating onto XLT4 agar. D-values were calculated for each type of sample studied. A linear regression model was applied to predict the survival of bacterial cells, with a two-tailed t-test to estimate P-values for statistical differences between radiative doses.

Results: There was a significant reduction of *Salmonella* sp. due to the UV light exposure. D-values were statistically different from control samples (inoculated, not UVC LED treated). At 2 mW/cm^2 irradiance, D-values for SS and CB were 0.64 min (CI 0.46-1.03) and 11.79 min (CI 8.15-21.31), respectively (P≤0.001). At 4 mW/cm^2 irradiance, CB showed a D-value of 8.07 min (CI 5.84-13.04), and TF, 0.91 min (CI 0.82-1.02) (P≤0.001).

Significance: This preliminary research may allow development of a device for microbial control during food processing or food service environments.

P1-189 Reduction of *Salmonella* on a Meat-based Pet Kibble Using *Lactobacillus salivarius* (L28)

ADAM CASTILLO[1], David Campos[2], Jorge Franco[1] and Mindy Brashears[1]
[1]*Texas Tech University, Lubbock, TX,* [2]*Texas Tech University, Muleshoe, TX*

✦ ***Undergraduate Student Award Competitor***

Introduction: Pets carrying *Salmonella* in their feces may be a potential vehicle for contamination of the household environment, potentially leading to human illness. Additionally, the pet food itself can be consumed inadvertently by children and pose a direct risk to the consumer.

Purpose: To determine the effect of *Lactobacillus salivarius* (L28) on the reduction of *Salmonella* in dry dog food kibble.

Methods: A cocktail of *Salmonella* (Enteriditis, Newport, and Typhimurium) was inoculated into a chicken fat coating that was applied to each designated control and treatment sample to yield 10^6 cfu/g on the product. Each sample was divided into two portions of a control sample and lactic acid bacteria (LAB) treated sample. Treated samples received L28 at concentrations to achieve 10^6 cfu per 0.5 lb of kibble. After treatment, each portion was allowed to dry at room temperature for 4 hours. Pet kibble grab samples of 25 grams were collected and enumerated for *Salmonella* on XLD agar with a thin-layer overlay at 0, 4, 24, and 72 hours.

Results: At 0h, the *Salmonella* counts on the kibble were approximately 6.0 log CFU/g in control and L28 treated samples. After four hours, both the control and treatments decreased in *Salmonella*, but the treated showed a 1.47 additional log reduction in comparison to the control. At 72h, the control counts were log 2.8 CFU/g. Furthermore, *Salmonella* was under the limit of detection after at 72h of treatment with L28. The experiment was replicated three times and there was a statistical difference between the *Salmonella* counts on the control and LAB treated pet kibble products.

Significance: The results suggest that L28 may be used to inhibit *Salmonella* on pet food products, hence reducing the risk of salmonellosis in consumers.

P1-190 Presence of *Bla*$_{CTX-M-8}$ in *Salmonella* Infantis Isolated from Poultry at Slaughterhouse in Brazil

DANIEL MONTE[1], Quezia Moura[1], Andressa Mem[2], Nilton Lincopan[1] and Mariza Landgraf[1]
[1]*University of São Paulo, São Paulo, Brazil,* [2]*Federal University of Parana, Curitiba, Brazil*

❖ ***Developing Scientist Competitor***

Introduction: Besides being one of the major foodborne pathogens worldwide, *Salmonella enterica* can harbor genes coding for resistance to β-lactam antibiotics. Poultry production chain could contribute to disseminating these genes. Moreover, the emergence of *bla*$_{CTX-M}$ genes has increased in *S. enterica*due resistance to third-generation cephalosporins which is overused in food producing animals.

Purpose: The aim of this study was to investigate the presence of Extended-spectrum β-lactamases (ESBLs) genes of the CTX-M family in *S.* Infantis isolates recovered from poultry at slaughterhouse in southern Brazil.

Methods: As part of a local surveillance study conducted for resistance genes monitoring in poultry slaughterhouse, forty *S.* Infantis isolates were phenotypically investigated for the presence of ESBL resistance by Kirby-Bauer disc diffusion susceptibility and Etest. Phenotypically ESBL-positive isolates were screened by PCR and clonal relatedness was performed by PFGE. Furthermore, the *bla*$_{CTX-M-8}$ positive isolate was characterized by whole genome sequencing (WGS).

Results: Out of 40 *S.* Infantis isolates screened, 4 displayed ESBL phenotype. Three isolates (7.5%) harboring *bla*$_{TEM}$ were confirmed by PCR and WGS revealed the presence of *bla*$_{CTX-M-8}$ in only one isolate (2.5%). In addition, six isolates (15%) were multidrug resistant (MDR) and the disc diffusion method revealed eight resistance patterns. Regarding MLST, the ST of *S.* Infantis harboring *bla*$_{CTX-M-8}$ was ST32. The *bla*$_{CTX-M-8}$ was located on an approximately 97â€‰kb IncI1 plasmid. IncI1 plasmid was submitted to plasmid multilocus sequence typing and was assigned to ST113. The conjugation experiments were successfully obtained from the *bla*$_{CTX-M-8}$ -positive isolates. All isolates showed clonal relatedness based on PFGE.

Significance: In conclusion, we report in this study one ESBL-positive CTX-M-8 / ST32 and three ESBL-positive TEM-type. Although the frequency of ESBL genes were low in *S.* Infantis our findings underscore the wide spread of resistance markers on poultry production chain, reinforcing the need for continuous surveillance of resistance genes circulating in foods.

P1-191 Antimicrobial Effectiveness of Eugenol or Geraniol Alone or Combined against *Escherichia coli* O157:H7 and *Salmonella enterica* in Pineapple Juice Held at 4°C

Aubrey Mendonca[1], **EMALIE THOMAS-POPO**[1], Angela Shaw[1], Samuel Kiprotich[1], Floyd Woods[2] and Armitra Jackson-Davis[3]
[1]*Iowa State University, Ames, IA,* [2]*Auburn University, Auburn, AL,* [3]*Alabama A&M University, Normal, AL*

Introduction: Plant essential oils (EOs) and several of their components exhibit antimicrobial effectiveness against foodborne pathogens; however, their use at effective levels in foods is largely limited by negative sensory changes. Application of EO components in combination may result in lower effective antimicrobial concentrations.

Purpose: A study was performed to evaluate the antimicrobial efficacy of eugenol or geraniol alone or combined against *Escherichia coli* O157:H7 and *Salmonella enterica* in refrigerated (4 ºC) pineapple juice.

Methods: Commercial pineapple juice (pH 3.4) containing 0.0 (control), 0.125, 0.25 or 0.50 µl/ml of each EO component (eugenol or geraniol) or dual-combinations containing 0.125 µl/ml of each component, were inoculated with 7.99 log CFU/ml of *E. coli* O157:H7 or 7.78 log CFU/ml *S.enterica*and stored at 4 ºC. Survivors were determined after 0, 1, 2, 4, 8 and 24 h by surface plating diluted (10-fold) juice samples on tryptic soy agar supplemented with 0.6% yeast extract and counting bacterial colonies after incubation (35°C, 48 h).

Poster

Results: No growth of either pathogen occurred in the refrigerated (4 ºC) juice with or without added antimicrobial. After 24 h, treatments with 0.50 µl/ml geraniol alone resulted in > 5 log CFU/ml reductions of both pathogens. Initial viable counts of *S. enterica* and *E. coli* O157:H7 in juice with 0.50 µl/ml geraniol decreased by ~ 7.78 log and 7.29 log, ($P < 0.05$) respectively, after 24 h; log reductions in juice with eugenol at 0.50 µl/ml were 3.45 (*S. enterica*) and 2.14 (*E. coli*O157:H7). Eugenol and geraniol combined (each at 0.125 µl/ml) eliminated ~ 1.91 log (*E. coli* O157:H7) and ~ 2.75 log (*S. enterica)* after 24 h.

Significance: Geraniol has good potential for killing enteric pathogens in pineapple juice.

P1-192 Effect of Essential Oils and Their Active Components on *Salmonella enterica* Serovar Newport- Inactivation or Induction into the Viable But Nonculturable (VBNC) State?

AISHWARYA RAO

University of Arizona, Tucson, AZ

❖ ***Developing Scientist Competitor***

Introduction: Previous studies have demonstrated the efficacy of plant-based antimicrobials against foodborne pathogens such as *Salmonella* Newport on organic leafy greens.

Purpose: It is believed that under stressful conditions, pathogens can enter into the VBNC state. Hence, there is a concern if antimicrobial exposure induces VBNC state in foodborne pathogens. The objective is to determine whether treatments with plant antimicrobials inactivate *Salmonella* or induce the state of VBNC.

Methods: *In vitro*, *S.* Newport culture was exposed to 0.1% of one of the three essential oils or their active components or phosphate buffered saline (PBS) for 0 and 3 h. In addition 10 grams of organic iceberg lettuce samples were inoculated with *S*. Newport and treated with 200 mL of 0.5% of one of the 3 essential oils or their active components in PBS for 2 min. The samples were stored at 4°C, and survivors enumerated on days 0, 1 and 3. Viability of the treated cells was determined using LIVE/DEAD® staining and viewing under a fluorescence microscope.

Results: *In vitro*, oregano oil and carvacrol demonstrated inactivation as the cells fluoresced red and no survivors were detected at both time points. Lemongrass oil and citral showed few green cells but exhibited a time-dependent reduction of 2.9-5.2 logs and 3.5-8.5 logs by 3 h, respectively. Cinnamon oil and cinnamaldehyde showed more green than red cells and had about 2.1-5 and 1.8-5 log reductions by 3 h, respectively. On iceberg lettuce, all treatments demonstrated a 2-3 log reduction in *Salmonella* population on Days 0 and 1 with no survivors detected by Day 3, in comparison to PBS that had survivors. The viability assay indicated corresponding inactivation/viability. The results showed inactivation of *Salmonella* (no induction of VBNC) by plant antimicrobials.

Significance: The results could provide the organic produce industry with natural sanitizers that are effective in killing *Salmonella*.

P1-193 Efficacy of *Jatropha curcas* Plant Extract against the Survival of *Salmonella* Enteritidis

AUTUMN WOODS[1], Armitra Jackson-Davis[2], Ernst Cebert[3], Arthur Hinton, Jr.[4] and Lamin Kassama[3]

[1]Alabama A&M University, Huntsville, AL, [2]Alabama A&M University, Madison, AL, [3]Alabama A&M University, Normal, AL, [4]Poultry Microbiological Safety and Processing Unit, U.S. National Poultry Research Center, Athens, GA

Introduction: The use of plant-derived antimicrobials has shown to be effective at inhibiting microbial growth. Although *Jatropha curcas* is known to possess antimicrobial properties, its efficacy against *Salmonella* Enteritidis has not yet been investigated.

Purpose: The purpose of this study was to evaluate the effectiveness of various concentrations of *Jatropha curcas* plant extracts against *S.* Enteritidis in a broth system.

Methods: The efficacy of various concentrations of *J. curcas* (10, 12.5, 25, 50, 60 and 100 mg/mL) against the survival of *S.* Enteritidis was determined. Plants were separated into stem, leaf, and root parts, and freeze-dried. Extracts were prepared by soaking 5 g of the ground plant material in 50 ml of methanol or water. The antimicrobial activity of the extracts were determined by observing growth *S.* Enteritidis in media supplemented with extracts in a Bioscreen C Microbiology Analyzer for 24 hr 35°C at 600 nm. The control consisted of *S.* Enteritidis without the presence of any extract.

Results: The results of the Bioscreen analysis indicate that *J. curcas* does have some antimicrobial abilities against *S.* Enteritidis. ANOVA analysis revealed a significant difference between the methanol and water based stem and root extracts at each of the various concentrations ($P < 0.05$). According to Fishers LSD test, only leaf/water extract at 50 mg/ml was significantly different ($P < 0.05$) from all other leaf extracts (water and methanol). Observed optical density values (average = 0.507) demonstrated that the various concentrations of extracts were lower than that of the control (1.229).

Significance: The ability of *J. curcas* to inhibit the growth of *Salmonella* Enteritidis indicates that these extracts could be used during post-harvest cleaning and sanitizing operations by incorporating the use of this plant derived antimicrobial as a sanitizer.

P1-194 Cinnamaldehyde Enhances the Killing Effect of High-pressure Processing against *escherichia coli* O157:H7 and *Salmonella Enterica* in Refrigerated (4°C) Carrot and Berry Juices

David Manu, **AUBREY MENDONCA**, Aura Daraba, James Dickson, Joseph Sebranek, Angela Shaw and Alan DiSpirito

Iowa State University, Ames, IA

Introduction: Continuous operation of high pressure processing (HPP) equipment at 600 to 700 Mega Pascals (MPa) or higher to inactivate pathogens in foods can increase maintenance costs and decrease the life of pressure vessels. Simultaneous exposure of pathogens to a naturally derived antimicrobial and HPP might permit use of lower pressure treatments for effective microbial inactivation.

Purpose: A study was conducted to evaluate the efficacy of cinnamaldehyde (CA) combined with HPP to inactivate *Escherichia coli* O157:H7 and *Salmonella enterica* in carrot juice (CRJ; pH 6.3) and a berry juice (BRJ; pH 3.6).

Methods: Both juices each with added CA at 0 (control), 0.10, 0.15 or 0.25 µl/ml were each inoculated with *E. coli* O157:H7 or *S. enterica* to obtain ~7.0 $\log_{10}$CFU/ml. Juices were pressurized at 300 or 400 MPa for 60, 90 or 120 seconds at 4°C. Viability of pathogens in the juices were evaluated via serial dilution and plating of juice on appropriate selective agar before pressurization, after pressurization (within 1 h) and at set time intervals during storage (4°C) of the juices.

Results: Addition of CA to juices increased the sensitivity of the pathogens to HPP with *S. enterica* exhibiting a greater loss in viability than *E. coli* to the CA/HPP ($P < 0.05$). CA (0.25 µl/ml) combined with 400 MPa (60 s) inactivated *S. enterica* by more than 5.5-log cycles in CRJ, whereas *E. coli* was inactivated by only 2.26 log cycles ($P < 0.05$). In the more acidic BRJ, CA (0.15 µl/ml) with a lower pressure (300 MPa for 120 s) resulted in complete inactivation (negative enrichment) and greater than a 5-$\log_{10}$CFU/ml reduction of both pathogens.

Significance: Application of CA as low as 0.15 µl/ml combined with HPP can facilitate the use of lower pressures for effective inactivation of enteric pathogens in juices.

Poster

P1-195 Antibacterial Efficacy of Geraniol against *Escherichia coli* O157:H7 and *Salmonella enterica* in Carrot Juice and a Mixed Berry Juice Held at 4°C

AUBREY MENDONCA, David Manu, Fei Wang, Aura Daraba and Angela Shaw
Iowa State University, Ames, IA

Introduction: Growing consumer demand for foods that are free of synthetic preservatives has generated much interest among food processors to use naturally-derived antimicrobials for controlling foodborne pathogens.

Purpose: A study was performed to investigate the antimicrobial efficacy of geraniol, a plant-based extract, against *Escherichia coli* O157:H7 and *Salmonella enterica* in refrigerated (4 ºC) carrot juice (CRJ) and a mixed berry juice (MBJ).

Methods: Carrot juice (pH 6.3, 8.5°Brix) and MBJ (pH 3.6, 12.3°Brix) with added geraniol at 0.5 to 1.5 or 2.0 µl/ml were inoculated with a 5-strain mixture of *E. coli* O157:H7 or *S. enterica* to obtain an initial viable count of 5.08 $\log_{10}$ CFU/ml. Inoculated juices without geraniol served as control. Numbers of viable pathogens in the juices held at 4°C for 24 hours were monitored by surface plating diluted (10-fold) juice samples on sorbitol MacConkey agar (*E. coli* O157:H7) and xylose lysine tergitol 4 agar (*S. enterica*) and counting bacterial colonies after incubation (35°C, 48 h).

Results: None of the pathogens grew in the refrigerated (4 ºC) juices with or without added geraniol. In control juices, pathogen viability decreased by ~1.15 to 1.33 $\log_{10}$ CFU/ml after 24 h. Both pathogens were more sensitive to the antibacterial effects of geraniol in MBJ compared to CRJ. Initial viable counts of *S. enterica* and *E. coli* O157:H7 in CRJ with 2.0 µl/ml geraniol decreased by ~4.56 - and 4.25 log, respectively, after 24 h ($P < 0.05$). In contrast, just after 1 h, a lower concentration of geraniol (1.0 µl/ml) completely inactivated both pathogens (negative enrichment test) in MBJ ($P < 0.05$).

Significance: Geraniol has good potential for use as a natural antimicrobial to destroy human enteric pathogens in refrigerated carrot or berry juice.

P1-196 Inhibition of *Salmonella* spp. and *Escherichia coli* by Lentil Protein Edible Films with Added Natural Antimicrobials

RAUL AVILA SOSA[1], Carlos Enrique Ochoa-Velasco[1], Addí Rhode Navarro-Cruz[1], Obdulia Vera-López[1], Paola Hernández-Carranza[1], Claudia Montalvo Paquini[2] and Martin Alvaro Lazcano-Hernandez[1]
[1]Benemérita Universidad Autónoma de Puebla, Puebla, Mexico, [2]Universidad Politécnica de Puebla, Puebla, Mexico

Introduction: New packaging materials are developed to increase potential applications by adding antimicrobial compounds. Lentils have the second-highest ratio of protein per calorie of any legume, after soybeans, and its protein can be used as a source of polymers to form edible films. Antimicrobial films have the potential to inhibit the growth of pathogens and deteriorative microorganisms present in foods.

Purpose: The aim of this work was to evaluate the inhibition of *Salmonella* spp. and *Escherichia coli* by lentil protein edible films with added natural antimicrobials.

Methods: Lentil protein concentrated was combined with water and glycerol to form edible film solutions; thymol and carvacrol were incorporated at different concentrations (0, 250, 500, 1000, and 1,500 mg/L). Minimal inhibitory concentration (MIC) was determined against *Salmonella* spp. and *E. coli* by the Kirby-Bauer method. To evaluate death curves, subletal concentrations were applied and evaluated by plate count method.

Results: Active edible films showed MIC values of 500 and 1500 mg/L for thymol and carvacrol, respectively, for *Salmonella* spp.; while *E. coli* appeared to be more sensitive to these compounds, with MIC values of 250 and 500 mg/L for thymol and carvacrol, respectively. For both microorganisms at 24 hours, subletal concentrations increased the lag phases and decreased the growth rates.

Significance: These results can help us understand how antimicrobials incorporated into protein edible films can inhibit foodborne pathogens.

P1-197 Antimicrobial Activity of White Mustard Essential Oil on *Salmonella* spp. in Vitro and in Ground Chicken

ADAM PORTER and Emefa Monu
Auburn University, Auburn, AL

❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* remains the leading cause of foodborne illness by a bacterial pathogen in the U.S. Preventive measures are needed in order to make food safer by inhibiting the growth of this pathogen. Natural antimicrobials have shown potential against microorganisms while meeting current consumer needs of less artificial preservatives and products.

Purpose: In this study, the antimicrobial activity of WMEO and carvacrol were evaluated against *Salmonella* spp. in tryptic soy broth and in ground chicken.

Methods: Five serovars of *Salmonella* (Enteritidis, Typhimurium, Heidelberg, Senftenberg, Hartford, and Orion) individually and in a cocktail were exposed to WMEO concentrations of 0.84%, 0.42% and 0.21% at 22°C for 24h in tryptic soy broth (TSB) using the macrodilution method. In addition a five strain cocktail of nalidixic acid resistant *Salmonella* serovars (Enteritidis, Typhimurium, Heidelberg, Montevideo and Kentucky) were inoculated to achieve a total population of 10^5 CFU/g in triplicate 100 g ground chicken samples with 7 treatments (positive control, un-inoculated control, 0.75% WMEO, 0.5% WMEO, 0.75% WMEO with 0.1% carvacrol, 0.5% WMEO with 0.1% carvacrol, and 0.1% carvacrol alone). Samples were plated every 2 days on TSA with 100ppm nalidixic acid over a storage period of 12 days at 4°C and 10°C.

Results: Treatment with 0.84% WMEO decreased populations of *Salmonella* spp. in TSB by 2-4 log CFU/mL, while 0.42% WMEO had a bacteriostatic effect and 0.21% slowed the growth rate. There was no difference ($P > 0.05$) in sensitivity between serovars. In chicken, WMEO alone and in combination with carvacrol decreased *Salmonella* counts by approx. 1 log, while carvacrol alone had no effect.

Significance: Results indicate the potential use of WMEO in the control of *Salmonella* spp. due to its consistent antimicrobial effect between serovars. It provides a possible preventive measure for the food industry to control *Salmonella* spp. in food.

P1-198 Essential Oil Nanoemulsions as Post-harvest Wash Solutions on Snacking Peppers

Laurel Dunn, Marion Harness, Dara Smith, Stuart Gorman, **ALEXIS HAMILTON**, P. Michael Davidson, Qixin Zhong and Faith Critzer
University of Tennessee, Department of Food Science, Knoxville, TN

Introduction: Consumer-driven demand for organic produce has resulted in increased research on alternative post-harvest wash solutions to control pathogens. Chlorine is a widely used compound due to low cost and antimicrobial efficacy. However, consumers and organic growers are interested in naturally sourced alternatives, such as essential oils, not only to reduce cross-contamination in a wash system, but also to inactivate attached pathogens, something chlorine does not readily do.

Purpose: This study was conducted to determine the antimicrobial efficacy of emulsified clove bud oil (CBO) or thyme oils (TO) as a post-harvest wash for snacking peppers inoculated with *Salmonella* spp.

Methods: Oils were saponified and emulsified with gum arabic prior to dilution in wash water. Snacking peppers were spot inoculated with a five-serovar cocktail of *Salmonella* from human outbreaks. Peppers were washed for 2 min with water, 200 ppm chlorine, 0.2% or 0.5% CBO, 0.2% or 0.5% TO, or received no rinse (control). All treatments were applied after the addition of 1% pepper puree, which simulated organic loading of wash water with continuous use. Uninoculated peppers were subsequently washed in used treatment solutions to show if cross-contamination could be prevented. After washing, *Salmonella* was enumerated from inoculated and uninoculated peppers. Wash solutions were filtered (0.45 µm) for enumeration.

Results: On inoculated peppers, 0.5% TO (± organic loading) and 0.5% CBO resulted in up to 1 log CFU/g reductions in *Salmonella* spp. ($P < 0.05$). Chlorine, 0.5% CBO (± organic loading), and both 0.2% and 0.5% TO (± organic loading) prevented attachment by *Salmonella* to clean peppers to less than the limit of detection (-1 log CFU/g). No detectable *Salmonella* were recovered in wash solutions containing chlorine and 0.2% CBO (without organic loading), all TO treatments, and CBO at 0.5% (± organic loading).

Significance: Nanoemulsified essential oils may be suitable for use in organic produce wash systems to limit cross-contamination.

P1-199 Antibiotic-resistant *Salmonella* spp. from Flies of Cattle Source

YUMIN XU[1], Sha Tao[2], Mark Harrison[3] and Jinru Chen[4]

[1]University of Georgia, Griffin, GA, [2]University of Georgia, Tifton, GA, [3]University of Georgia, Athens, GA, [4]University of Georgia, Department of Food Science and Technology, Griffin, GA

❖ ***Developing Scientist Competitor***

Introduction: Mostly, human salmonellosis is caused by consuming foods that originated from animals that are reservoirs of *Salmonella* spp., such as cattle. Flies can be transmitters of *Salmonella* spp. from cattle to humans. Antibiotic resistant *Salmonella* from cattle, due to extensive use of antibiotics on cattle farm, can be potentially disseminated to humans by flies. Integrons are considered a critical source of antibiotic resistance genes that can be widely spread by horizontal gene transfer to aggravate the antibiotic resistant issue of *Salmonella*.

Purpose: The purpose of this study was to identify the prevalence of *Salmonella* in flies captured from cattle farms and to screen for the presence of integrons among isolated *Salmonella* spp. strains.

Methods: Flies (50 from each farm) were captured using fly tapes on 33 cattle farms interspersed throughout Georgia, U.S.A., from June 2016 to September 2016. *Salmonella* were isolated from the internal surface and external tissue of the flies following a method outlined in FDA Bacteriological Analytical Manual with slight modification. Presence of integrons among isolated *Salmonella* spp. strains were screened using PCR with primers derived from the integrase gene.

Results: *Salmonella* spp. were isolated from 26 out of 33 cattle farms, where 185 out of 1,650 flies (11.2%) were found *Salmonella*-positive. The incidence of *Salmonella*-positive flies varied from farm to farm, ranging from 0% to 78%. The top three incidences were 78%, 52%, and 30%. Integron positive *Salmonella* were found in 3 out of 33 sampled farms (9.1%).

Significance: These data suggest that flies are active carriers of *Salmonella* on cattle farms. However, the incidence of *Salmonella*-positive flies varied depending on herd size, environmental hygiene, and farm management. Antibiotic resistance genes were not commonly found in the *Salmonella* isolated from the cattle farms in Georgia.

P1-200 Slow-release Chlorine Dioxide Gas Treatment to Reduce *Salmonella* Contamination on Spices for Small-scale Processors

CHASE GOLDEN[1], Mark Berrang[2], William Kerr[1] and Mark Harrison[1]

[1]University of Georgia, Athens, GA, [2]U.S. Department of Agriculture-ARS-USNPRC, Athens, GA

❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* is a major food safety concern in spices. Multiple studies have shown chlorine dioxide's (ClO_2) effectiveness at reducing microbial levels on produce and other food products. Spices treated with ClO_2 generated from self-contained sachets could be a potential antimicrobial treatment for small-scale processors and distributors.

Purpose: This study determined the effectiveness of a slow-releasing ClO_2 gas generated from self-contained sachets to reduce *Salmonella* contamination on spices and to see if this treatment would be applicable on a small-scale. The combined effect of ClO_2 gas treatment and storage time on *Salmonella* levels on spices was evaluated.

Methods: Three different spices (black peppercorns, sesame seeds, and cumin seeds) were inoculated with a cocktail of five different nalidixic acid resistant *Salmonella* strains by soaking and mixing the spices in the cocktail for 30 min. The inoculated spices were then dried in a biosafety hood for 24 h. Spices were treated with 0, 100, 200, or 500 mg ClO_2/kg spice using a self-contained, slow-release ClO_2 media in a rotating tumbler for 12 h. Immediately after treatment, and at days 1, 10, and 30 post-treatment, samples were plated to enumerate surviving *Salmonella*.

Results: When compared to the control treatment, each concentration of ClO_2 was effective in reducing *Salmonella* contamination for all three spices tested. Out of the three different concentrations of ClO_2 tested, 500 mg/kg decreased *Salmonella* levels by > 2.0 logs at day 0. *Salmonella* levels stayed relatively consistent across the first 10 days of post-treatment storage, but levels decreased by at least an additional 0.5 logs by day 30.

Significance: Chlorine dioxide gas provides a quality, non-heat alternative treatment, especially for smaller-scale operations to reduce *Salmonella* contamination in spices. The versatility of being able to use the treatment at many points in the supply chain also makes it desirable.

P1-201 SalmoFresh™ Effectiveness as a Bio-control Method to Eliminate *Salmonella* Prevalence on Romaine Lettuce, Mungbean Sprouts, and Mungbean Seeds

XUAN ZHANG[1], Yan Dong Niu[2], Kim Stanford[3], Richard Holley[1], Tim McAllister[4] and Claudia Narvaez[1]

[1]University of Manitoba, Winnipeg, MB, Canada, [2]Alberta Agriculture and Forestry, Lethbridge, AB, Canada, [3]Alberta Agriculture, Lethbridge, AB, Canada, [4]Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

Introduction: Produce commodities have been linked to *Salmonella* outbreaks worldwide. Purified bacteriophages such as SalmoFresh™ have been successfully applied to control *Salmonella* in food.

Purpose: To assess SalmoFresh™ effectiveness to reduce/eliminate *Salmonella* on romaine lettuce, mungbean sprouts and mungbean seeds.

Methods: SalmoFresh™ effectiveness was tested against five *Salmonella* strains (Newport, Braenderup, Typhimurium, Kentucky and Heidelberg) at exponential growth individually, at 2, 10 and 25 ºC using the microplate virulence assay in duplicate. Lettuce (n=98) (33cm^2 pieces), mungbean sprouts (n=98) and mungbean seeds (n=40) were washed with tap and chlorinated water (150 ppm for lettuce & sprouts; 1000 ppm for seeds) and rinsed twice

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with non-chlorinated water before inoculation with *Salmonella* cocktail (10^6 CFU/ml). SalmoFresh™ (10^8 PFU/ml) was sprayed on lettuce & sprouts and held for 1, 24, 48 and 72 h (2, 10 and 25 ºC). Seeds were exposed to the phage by immersion (1 h at 25 ºC). *Salmonella* reduction/survival was determined by total plate count (XLT4 agar) and immunomagnetic separation. Mungbean seeds from control and treatment groups were germinated without treating by SalmoFresh™, and *Salmonella* prevalence was re-examined.

Results: Microplate assay results indicated that SalmoFresh™ (10^8 PFU/ml) reduced (P=0.007) *Salmonella* by an average of 5 logs CFU/ml during the first 5 h. However, the spot plate technique showed that some *Salmonella* survived after 24 h (37°C). *Salmonella* was reduced by 1.5 log CFU/g and 1.65 log CFU/g in lettuce and sprouts, respectively. Reduction on seeds was only 0.2 logs CFU/g.

Significance: Chlorinated water and SalmoFresh™ alone or in combination were not sufficient to control *Salmonella* contamination on fresh produce, especially in seeds where reductions were minimal.

P1-202 Antimicrobial Activity of Curcumin Under UVA Light Radiation: Application to Fresh Produce Sanitation

ERICK FALCAO DE OLIVEIRA[1], Juliano Tosati[2], Andrea Cossu[1], Rohan Tikekar[3], Alcilene Monteiro[2] and Nitin Nitin[1]

[1]University of California-Davis, Davis, CA, [2]Universidade Federal de Santa Catarina, Florianopolis, Brazil, [3]University of Maryland, College Park, MD

❖ ***Developing Scientist Competitor***

Introduction: Reducing microbial contamination is critical for the safety and quality of fresh produce. Numerous foodborne outbreaks linked to fresh produce illustrate the unmet need to improve sanitation methods. Consumer demand for reducing the use of conventional sanitizers, such as chlorine, in fresh produce and stringent environmental regulations motivates development of food-grade biocidal agents.

Purpose: This work evaluated the synergistic antimicrobial activity of a food-grade compound (curcumin) with UV-A light radiation.

Methods: Bacterial samples were treated with curcumin and exposed to UV-A light under different experimental conditions (i.e. curcumin concentration, UV-A light radiation time, pH, temperature, chemical oxygen demand). After treatment, microbial counts were determined by the plate counting method.

Results: The antimicrobial activity of photo-activated curcumin was dependent on the curcumin concentration. Treatments with five ppm of curcumin were able to reduce the bacterial load from 6 log CFU/mL to levels below the limit of detection (1 log CFU/mL). Furthermore, the antimicrobial activity was significantly enhanced at lower pH ($P < 0.01$); but, not influenced by treatment at room and refrigerated temperatures ($P > 0.05$). Even though increased chemical oxygen demand (COD) in solution reduced the antimicrobial activity of photo-activated curcumin, five log CFU/mL microbial inactivation was observed for bacteria in the presence of high COD (1000 ppm). During simulated spinach washing, a five minute treatment with curcumin + UVA light lowered the bacterial count in solution from five log CFU/mL to levels below the detection limit of one log CFU/mL. In addition, cross-contamination to spinach leaves was significantly reduced as compared to UV-A light by itself ($P < 0.01$).

Significance: In summary, this study illustrates the potential of combining the food-grade compound curcumin and UV-A light to achieve bacterial load reduction in fresh produce sanitation.

P1-203 Inhibitory Effects of *Mentha piperita* L. Essential Oil against *Escherichia coli* O157:H7 and *Salmonella* Enteritidis PT4 in Fruit Juices

MARCIANE MAGNANI[1], Rayssa Juliane de Carvalho[2], Larissa de Fátima Romão da Silva[2], Maísa Gomes Chaves[2], Evandro Leite de Souza[3] and Geany Targino de Souza[2]

[1]Federal University of Paraiba, Joao Pessoa, Brazil, [2]Federal University of Paraiba, João Pessoa, Brazil, [3]Federal University of Paraíba, João Pessoa, Brazil

Introduction: Outbreaks of foodborne disease have been associated with raw juices. Regulations in the United States require the application of treatments that result in a five-log reduction of the pertinent pathogens in juices. *Mentha piperita* L. essential oil (MPEO) has been cited as a safe antimicrobial for drinks; however, its inhibitory effects against pathogens in juices remains unknown.

Purpose: This study was conducted to evaluate the inhibitory effects of MPEO against *Escherichia coli* O157:H7 and *Salmonella* Enteritidis PT4 in juices of caja, guava, and mango.

Methods: Samples of juice (3600 µL) containing MPEO at 5 µL/mL (minimal inhibitory concentration) or 2.5 µL/mL were inoculated with 400 µL of bacterial suspension (10^7 CFU/mL) and incubated at 8-10°C. The number of viable cells (log CFU/mL) was counted at 0, 1, 2, 4, 8, 12 and 24 h by serial dilution and plating on Eosine-Methylene-Blue agar (*E. coli* O157:H7) or Xylose-Lysine-Deoxycholate agar (*S*. Enteritidis PT4). Juices without MPEO were assayed similarly (controls). Statistical analysis was performed in Sigma Stat 4.0 (P<0.05).

Results: MPEO at 5 µL/mL caused a 5-log reduction of both strains after 4 h in caja juice and after 12 h in guava juice. In mango juice, at this concentration, MPEO reduced 5 log units of *E. coli* O157:H7 and *S.* Enteritidis PT4 after 12 h and 24 h, respectively. MPEO at 2.5 µL/mL caused 5-log reduction of *E. coli* O157:H7 and *S.* Enteritidis PT4 after 8 h and 12 h, respectively in caja juice. In guava and mango juices, MPEO at 2.5 µL/mL reduced approximately 4.5 log units of both strains after 24 h. No reductions in viable counts of test strains were observed in controls over 24 h.

Significance: MPEO may inhibit pertinent pathogens in raw juices; however, the concentration used for inactivation should be carefully evaluated to guarantee the microbiological safety in these products.

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P1-204 Antimicrobial Properties of High Molecular Weight, Water Soluble Chitosan in Gram Negative Foodborn Pathogens

NANCY RUBIO, Rita Quintero, Jose Fuentes, Marlene Janes and Witoon Prinyawiwatkul

Louisiana State University, Baton Rouge, LA

❖ ***Developing Scientist Competitor***

Introduction: Chitosan is an abundant sub-product of the seafood industry that has antimicrobial activity against foodborne pathogens.

Purpose: The intent of this study was to determine the antimicrobial properties of chitosan, using a new fast dissolving high molecular weight chitosan, against *Escherichia coli* O157:H7 (*Ec*), *Salmonella* Typhimurium (*ST*), *Vibrio cholera (Vc)* and *Vibrio vulnificus (Vv)*.

Methods: Our patent pending high molecular weight, water-soluble chitosan, with molecular weights of 789 kDa and 1017 kD, were dissolved in acetic acid (AC) at 1% w/v or aspartic acid (AS) at 1-4% w/v. Chitosan samples (1 ml) were added to 8 ml of Mueller Hinton broth, then inoculated with 1 ml of 12 h old bacteria cultures to give an initial inoculation of >6.50 log CFU/g. All samples were incubated at 25°C and bacterial counts were determined at 0, 48, and 96 h, by plating onto Mueller Hinton agar. Plates were incubated for 48 h at 37°C and Log CFU/ml determined.

Results: Chitosan treatments 789AC1%, 789AS1%, and 1017AC1% reduced *Vc and Vv* counts to non-detectable levels after 48 h at 25°C. *Ec* bacterial counts were reduced by 2 Log CFU/ml when treated with 789AC1%, 1017AC1%, 789AS2%, and 789AS4%. Only one chitosan treatment significantly reduced *S*T by 2 Log CFU/ml (789AC1%) from control levels.

Significance: Our results found chitosan 789AC1% was the most effective antimicrobial treatment significantly reducing *Ec*, *S*T, *Vc*, and *Vv* bacterial counts from the control levels.

P1-205 Determination of the State of *Escherichia coli* O157:H7 Cells Treated with Electrolyzed Oxidizing (EO) Water Using Flow Cytometry

G. KWABENA AFARI and Yen-Con Hung
University of Georgia, Griffin, GA

❖ ***Developing Scientist Competitor***

Introduction: Electrolyzed oxidizing (EO) water has been shown to be highly effective in the reduction of microorganisms on foods. Concerns, however, persist that this may not lead to complete inactivation of pathogens, but results in a proportion of cells entering the viable but non-culturable (VBNC) state, which can cause serious food safety concerns.

Purpose: This study was conducted to determine the lethality of EO water treatments on *Escherichia coli* O157:H7 cells and proportions of cells that enter the VBNC state using flow cytometry and plating.

Methods: A 5-strain cocktail of *E. coli* O157:H7 (approx. 6 log CFU/mL) was exposed to varying free chlorine concentrations (2.5, 5, 10 mg/L) of near neutral EO (NEO) water, acidic EO (AEO) water and NaOCl solutions for 30, 60, and 120 s. Sodium thiosulfate was added as a neutralizing solution, after treatment, and cells were recovered on plates of Sorbitol MacConkey agar supplemented with nalidixic acid and sodium pyruvate, as well as Tryptic Soy enrichment broths. For VBNC determination, 3 µL of a mixture of propidium iodide and SYTO™9 dyes was added to 1 mL of each treated sample and subsequently analyzed in a flow cytometer. Deionized water treatments served as controls and experiments were replicated three times.

Results: Sanitizer type had a significant effect ($P<0.05$) on the reductions of *E. coli* O157:H7 observed on plates, with AEO water treatments resulting in complete inactivation of pathogens after enrichment (> 6 log CFU/mL reduction). For NEO water, growth was, only, observed after enrichment. In the case of NaOCl, longer contact times were needed to achieve significant reductions. Up to 4% of cells remained in the live region of flow cytometric profiles of NEO water and NaOCl treated cells.

Significance: Results suggest that although EO water treatments lead to great reductions in *E. coli* O157:H7 cells, some cells may enter the VBNC state.

P1-206 Development of Predictive Reduction Models for *Escherichia coli* as a Function of Sodium Dichloroisocyanurate and Chlorine Dioxide Concentration and Exposure Time

SO-JEONG YOON[1], Shin Young Park[1], Kye-Hwan Byun[1], Hyung-Suk Kim[1], Yong-Soo Kim[2] and Sang-Do Ha[1]
[1]Advanced Food Safety Research Group, Brain Korea 21 Plus, Chung-Ang University, Ansung, South Korea, [2]KHIDI, Cheongju, South Korea

Introduction: There is increasing concern regarding misusing disinfectants and sanitizers such as ethanol, sodium hypochlorite, and hydrogen peroxide for food contact surfaces in the food industry. Examining the efficacy of the concentration of currently used disinfectants and sanitizers is urgently required in the Korean society.

Purpose: This study aimed to develop predictive reduction models for *Escherichia coli* in suspension, as a function of sodium dichloroisocyanurate (SDIC), chlorine dioxide (CD), and exposure time using response surface methodology.

Methods: Under clean condition (bovine albumin 0.3g/L), *Escherichia coli* ATCC 10536 (initial inoculum, 8-9 $\log_{10}$CFU/mL) in tryptic soy broth was treated with different concentrations of SDIC (100 and 200 ppm) or CD (5, 20, and 35 ppm) for different exposure times (0.5, 2.5, and 5 min for SDIC or 1, 3, and 5 min for CD) following a central composite design. The polynomial reduction models for SDIC or CD on *E. coli* were used under the clean condition.

Results: *Escherichia coli* reduction by 200 ppm SDIC for 0.5, 2.5, and 5 minutes was 0.92, 1.65, and 4.36 $\log_{10}$CFU/mL, respectively. Also, *E. coli* reduction by 35 ppm CD for 1, 3, and 5 minutes was 2.49, 2.70, and 3.65 $\log_{10}$CFU/mL, respectively. The predictive response surface quadratic polynomial models developed were $Y=0.28275-0.013382X_1-0.11310X_2+3.44500E^{-003}X_1X_2+8.16214E^{-005}X_1^2$ ($R^2=0.90$) for SDIC and $Y=0.43231-0.056492X_1-0.097771X_2+9.24167E^{-003}X_1X_2+3.06333E^{-003}X_1^2$ ($R^2=0.99$) for CD, where Y was the bacterial reduction ($\log_{10}$CFU/mL), X_1 was the concentration and X_2 was the exposure time.

Significance: Our predictive reduction models should be validated in developing the optimal concentration and exposure time of disinfectants and sanitizers for inhibiting *E. coli* on food contact surfaces in the food industry.

P1-207 Antimicrobial-resistance Patterns of Generic *Escherichia coli* Isolated from Feedlot Cattle Feces after Feeding Direct-fed Microbials in Diets with and without Tylosin during Finishing

ANDREA ENGLISH, Alejandro Echeverry, Jhones Sarturi, Kendra Nightingale, Tosha Opheim, Mark Miller and Mindy Brashears
Texas Tech University, Lubbock, TX

Introduction: Properly screened, direct-fed microbials (DFM) may be an effective alternative to the use of antibiotics (tylosin) in cattle diets and may help reduce the emergence and dissemination of antimicrobial resistance (AMR) in feedlot cattle.

Purpose: The purpose of this study was to determine the AMR patterns of generic *Escherichia coli* isolated from feedlot cattle fed stem-flaked, corn-based diets supplemented with either DFM (*L. salivarious* L28) or tylosin.

Methods: A newly isolated DFM, *L. salivarious* L28, was used in this study. Three treatments based on conventional high concentrate diets were fed to finish cattle for harvest: base (no DFM, tylosin, or monensin), MonPro (DFM with monensin, but no tylosin), and a control (tylosin and monensin). A total of 36 composite fecal samples from beef cattle feedlot pens, housing 3 animals per pen, were collected after 56 days of feeding each treatment. Samples were weighed, enriched, and plated onto MacConkey agar, and three isolates were randomly selected and streaked onto blood agar plates for antimicrobial resistance analysis using Sensititre™ susceptibility MIC plates, following the National Antimicrobial Resistance Monitoring System (NARMS) protocol.

Results: Generic *E. coli* was isolated from 100% ($n=36$) of fecal samples collected. After 56 days of feeding, 61% ($n=22$) of the control group isolates were resistant to at least one antibiotic and 11% ($n=4$) were multi-drug resistant (MDR) to three or more antibiotics. Isolates from the base treatment group, on day 56, exhibited 56% ($n=20$) resistance to one drug and 19% ($n=7$) were MDR. Thirty three percent of isolates in the MonPro group showed resistance on day 56, where only 6% ($n=2$) of isolates were MDR.

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Significance: With the recent restrictions of antibiotic use (tylosin) in cattle feeding operations, this study suggests that using the DFM, *L. salivarious* L28, may help reduce the rates of emergence of AMR to *E. coli* in cattle.

P1-208 Polyphenolic Compounds Alter Viability, Swarming Motility and Biofilm Formation of Pathotypes of *Escherichia coli*

CAROLINA GIL[1], Santos Garcia[1], Norma Heredia[1], Jorge Dávila-Aviña[2] and Luisa Solís-Soto[2]

[1]Universidad Autonoma de Nuevo Leon, San Nicolas, Mexico, [2]Universidad Autonoma de Nuevo Leon, San Nicolas de los Garza, Mexico

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Introduction: Pathotypes of *E. coli*are common cause of diarrhea. Phenolic compounds have been reported as good alternatives to improve food safety due to their high antimicrobial activity.

Purpose: To evaluate the activity of polyphenolic compounds on growth, viability, swarming motility and biofilm formation index (BFI) of pathotypes of *E. coli* (EPEC, EHEC and ETEC).

Methods: Minimum bactericidal concentrations (MBCs) of tannic acid (TA), gallic acid (GA), methyl gallate (MG) and epigallocatechin gallate (EGCG) were evaluated by a microdilution method. The effect of sublethal concentrations (SC) on growth and viability was assessed by the pour plate method, and cell membrane integrity by flow cytometry. Swarming motility was determined in soft LB agar. BFI was determined by a colorimetric method.

Results: The MBCs of polyphenolic compounds against three pathotypes of *Escherichia coli,* showed values between 80-2500 µg/ml. MG was the most effective to inhibit growth of all tested bacteria (at concentrations of 80-600 µg/ml), while TA was the less effective (1800-2400 µg/ml). EPEC was the most susceptible (80-2100 µg polyphenols/ml), while ETEC was the most resistant (600-2400 µg polyphenols/ml). SC of compounds did not affect bacterial growth. All compounds at SC reduced swarming motility by 20%-100%. TA exhibited the highest reduction of swarming motility at 1250 mg/mL (100%) for EHEC and ETEC. Most compounds reduced BFI, but at various concentrations it was induced. GA produced the greatest inhibitory effect on BFI of EHEC and ETEC. TA induced BFI of EPEC, while MG and EGCG induced BFI of EHEC and ETEC.

Significance: All polyphenols showed bactericidal activity against *E. coli* of three pathotypes, although SC had different effects on phenotypic traits, and various compounds increased the expression of virulence factors.

P1-209 Effectiveness of Individual and Combined Antimicrobial Spray Interventions Commonly Used on Chilled Beef Subprimals

JENNIFER ACUFF[1], Matthew Krug[1], Daniel Vega[1], Nicholas Sevart[1], Sarah Jones[1], Amanda Wilder[1], Keyla Lopez[1], Minto Michael[1], Christopher Vahl[2], Gary Acuff[3], Harshavardhan Thippareddi[4] and Randall Phebus[1]

[1]Kansas State University, Manhattan, KS, [2]Kansas State University, Department of Statistics, Manhattan, KS, [3]Texas A&M University, College Station, TX, [4]University of Georgia, Athens, GA

Introduction: Beef processors use interventions throughout the slaughter and fabrication process to reduce Shiga toxin-producing *Escherichia coli* (STEC) contamination risks. Certain approved antimicrobials are marketed for spraying chilled subprimals; however, validation of these treatments using commercial-scale equipment and foodborne pathogens is lacking.

Purpose: This study evaluated the efficacy of three common antimicrobial sprays, individually and combined, against a rifampicin-resistant STEC cocktail (O26, O45, O103, O111, O121, O145, and O157:H7).

Methods: For individual antimicrobial treatments, beef subprimals (n=16) were mist-inoculated with the cocktail (ca. 5 log CFU/cm^2), followed by spray-treatment with 200 ppm peracetic acid (PAA), 2% Centron™ (sulfuric acid, sodium sulfate anhydrous and water mixture; CEN), 4.5% lactic acid (LA), or water (W). For combined treatments, inoculated subprimals (n=4) were first treated with PAA, LA, CEN, or W; vacuum packaged; and stored for 72 hours at 4°C. Each subprimal was then divided (n=16) and treated with each of the four antimicrobials as a second treatment. The first study was designed as randomized generalized block, and the second study was designed as a split plot. After each treatment phase, microbial analyses were conducted in duplicate to determine STEC reductions.

Results: For individual antimicrobial treatments, LA and PAA provided greater (P≤0.05) STEC reductions (0.5 and 0.6 log CFU/cm^2, respectively) compared to water (0.1 log CFU/cm^2), but the CEN reduction (0.2 log CFU/cm^2) was similar to water. For combined treatments, reductions ranged from 0.5 log CFU/cm^2 to 1.5 log CFU/cm^2; the greatest reduction observed when subprimals were treated with LA, vacuum packaging, and another LA application.

Significance: These studies indicate that the individual antimicrobial treatments evaluated are marginally effective for reducing STEC population on chilled beef subprimals during fabrication. Their efficacy may be improved by combining treatments when the beef is stored under vacuum packaged conditions and retreated upon bag opening, as typical of mechanical tenderization operations.

P1-210 Efficacy of an Ambient Water Wash, Hot Water Wash, and Application of Three Antimicrobial Sprays Using a Three-stage Commercial Carcass Washing Cabinet for Reducing Shiga Toxin-producing *Escherichia coli* Contamination on Beef Carcasses

MATTHEW KRUG[1], Jennifer Acuff[1], Nicholas Sevart[1], Minto Michael[1], Daniel Vega[1], Christopher Vahl[2], Gary Acuff[3], Harshavardhan Thippareddi[4] and Randall Phebus[1]

[1]Kansas State University, Manhattan, KS, [2]Kansas State University, Department of Statistics, Manhattan, KS, [3]Texas A&M University, College Station, TX, [4]University of Georgia, Athens, GA

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) associated with live cattle pose a risk for beef contamination during slaughter. To control these risks, beef carcass intervention methods are widely implemented across the industry.

Purpose: The antimicrobial effectiveness of intervention methods applied sequentially to pre-rigor beef carcass sides, using a three-stage commercial spray cabinet (Chad Equipment) to reduce STEC contamination, was evaluated.

Methods: Four pre-rigor carcass sides were inoculated by electrostatic spraying with a 7-serogroup STEC cocktail (ca. 6 log CFU/100 cm^2). The following treatments were applied, in order, to each side in the cabinet: ambient water wash, hot water wash, and antimicrobial mist. Each side was assigned one of four chemical treatments: no treatment (control), peracetic acid (200 ppm), lactic acid (4.5%), or Centron™ (1.1 pH). Excision samples were taken from the top, middle, and bottom region of each side at: 30 min post-inoculation, post-ambient water wash, post-hot water wash, post-antimicrobial spray, and after 18 h spray chilling (three replications conducted).

Results: The cabinet's ambient water stage reduced the STEC population on sides by 0.9, 1.1, and 1.5 log CFU/100 cm^2 and the hot water stage, additionally, by 2.6, 3.6, and 3.3 log CFU/100 cm^2 at the bottom, middle, and top of the carcass, respectively. Although the STEC reduction observed at

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the top of the carcass was higher ($P \leq 0.05$) than the bottom for ambient water, no differences ($P > 0.05$) among sampling locations were observed at the other sampling points. Minimal additional STEC reductions were obtained after chemical spray application and chilling, due to STEC contamination reaching very low or undetectable levels with the post-hot water wash.

Significance: Sequential antimicrobial treatments applied using a carcass wash cabinet (ambient and hot water washes, followed by approved chemical sprays) reduced STEC populations on pre-rigor beef carcasses by 4.5–5.3 log CFU/100 cm^2; significantly reducing STEC risks on fabricated beef products.

P1-211 Efficacy of Peracetic Acid Washes Applied at Increasing Concentrations to Control Shiga Toxin-producing *Escherichia coli* Contamination on Chilled Beef Subprimals

MATTHEW KRUG[1], Sarah Jones[1], Nicholas Sevart[1], Jennifer Acuff[1], Amanda Wilder[1], Minto Michael[1], Christopher Vahl[2] and Randall Phebus[1]

[1]Kansas State University, Manhattan, KS, [2]Kansas State University, Department of Statistics, Manhattan, KS

Introduction: Shiga toxin-producing *E. coli* (STEC) are bacteria commonly associated with foodborne disease outbreaks, especially attributable to beef. Evaluation of intervention methods is necessary to better control these pathogens in the beef industry.

Purpose: This study validated the efficacy of increasing concentrations of a peracetic acid applied as a spray (PAA; Microtox Plus, ZEE Company) prior to chilled subprimal packaging for reducing populations of STEC. Impact on raw beef color was documented.

Methods: Ten beef strip loins were inoculated to ca. 5 log CFU/cm^2 with a 7-serogroup (O157:H7, O26, O45, O103, O111, O121, O145) cocktail of *rifampicin*-resistant STEC. After 30-min attachment, subprimals were sprayed with PAA at increasing concentrations from 200–1800 ppm (at 200 ppm intervals) or a water control (0 ppm) before being vacuum packaged and stored for 24 h at 4°C. Excised tissue samples were taken from the top and bottom of each subprimal and microbiologically analyzed to quantify STEC reductions and L, a*, and b* color measurements were taken from the sides of the subprimals at three sampling points: post-inoculation, 5 min post-PAA spray, and post-24 h vacuum packaged chilling.

Results: Post-PAA treatments, STEC populations decreased by 0.5–1.3 log CFU/cm^2 across all concentrations, which were different ($P \leq 0.05$) than the water control (0.1 log CFU/cm^2 reduction). No differences ($P > 0.05$) were observed among application of concentrations between 400–800 ppm. STEC populations did not change ($P > 0.05$) in the PAA-treated subprimals after 24 h of chilled vacuum packaged storage. Although subprimal color changed over time, no difference ($P > 0.05$) was seen in color readings among PAA treatments of increasing concentrations.

Significance: These data suggest that a wide range of peracetic acid concentrations (≥ 400 ppm) will significantly reduce STEC on chilled beef subprimals, while having no effect on raw product color.

P1-212 Efficacy of Lactic Acid Washes Applied at Increasing Concentrations to Control Shiga Toxin-producing *Escherichia coli* Contamination on Chilled Beef Subprimals

MATTHEW KRUG[1], Ian Patterson[1], Nicholas Sevart[1], Jennifer Acuff[1], Minto Michael[1], Christopher Vahl[2] and Randall Phebus[1]

[1]Kansas State University, Manhattan, KS, [2]Kansas State University, Department of Statistics, Manhattan, KS

Introduction: Shiga toxin-producing *E. coli*(STEC) are frequently associated with foodborne illness outbreaks in beef products. Intervention methods, such as organic acid and/or other chemical blend sprays, are widely used to control enteric pathogen contamination. Quantifying the antimicrobial effectiveness of different concentrations of lactic acid (LA) against STEC is important for processors to optimize applications and support regulatory food safety plans.

Purpose: This study evaluated the efficacy of increasing concentrations of lactic acid (88%, Birko Corporation) to reduce STEC contamination on chilled beef subprimals when applied as a spray prior to vacuum packaging and after 24 h of wholesale bagged storage.

Methods: Beef strip loins (n= 12) were inoculated with a 7-serogroup STEC cocktail, (30 min attachment). Subprimals were sprayed with increasing concentrations of LA (3.0-10.0 % at 0.5% intervals) or a water control (0%). Meat surface excision samples and color readings (L*, a*, and b*) were obtained from each subprimal post-inoculation, 5 min after a LA spray, and after 24 h of vacuum packaged storage. Three replications were performed.

Results: LA spray reduced STEC contamination on subrimals by 0.2 – 0.7 log CFU/cm^2 (initial level of ca. 5 log CFU/cm^2). All LA concentrations except 3 % produced a higher population reduction ($P \leq 0.05$) compared to water. No differences ($P > 0.05$) were observed in reductions among LA concentrations of 3.5 -10 %. After chilled vacuum packaged storage, subprimal STEC populations did not change ($P > 0.05$). Application of higher concentrations of LA reduced ($P \leq 0.05$) L* and b* color values compared to the water control; similar results were observed after the 24-h chill period.

Significance: LA sprays ≥ 3.5% may reduce STEC contamination on chilled beef subprimals (by <1 log cycle), but application of higher concentrations provided similar reductions and may negatively impact product color.

P1-213 Prevalence and Mechanism of Fluoroquinolone Resistance in *Escherichia coli* Isolated from Swine Feces in Korea

KUN TAEK PARK, Yoon Sung Hu, Young Kyung Park, Sook Shin and Yong Ho Park

Seoul National University, Seoul, South Korea

Introduction: Fluoroquinolone (FQ) resistance is rapidly increasing, worldwide, and considered a serious threat to the public health. FQ has been prohibited as a feed additive since 2009 in Korea. Consequently, FQ-resistant bacteria are expected to decrease in the animal industry in Korea.

Purpose: This study was performed to investigate i) the prevalence of FQ resistance and plasmid mediated quinolone resistance (PMQR) genes in *Escherichia coli* isolated from swine, and ii) the antimicrobial resistance profile and FQ resistance mechanisms of FQ resistant *E. coli*.

Methods: *Escherichia coli* were isolated from 237 swine feces. FQ resistant *E. coli* were identified by the standard disk diffusion test and PMQR were detected by PCR. Antimicrobial susceptibility tests were performed against 16 different antimicrobial agents, and the 3 major FQ resistance mechanisms were investigated by sequencing of quinolone resistance determining regions (QRDR), detection of PMQR, and measuring of efflux pump activity.

Results: Of 171 *E. coli* isolates, 59 (34.5%) isolates were determined as FQ-resistant. Of 59 FQ-resistant isolates, PMQR genes were detected in nine isolates (15.3%). Efflux pump activity was found in 56 isolates (94.9%). Point mutation in QRDR was detected in all 59 isolates (100%) and determined to be the main cause of FQ resistance. Of 59 FQ-resistant *E. coli*, 54 isolates (91.5%) were classified as multi-drug resistant *E. coli* and 11 isolates (18.6%) were confirmed as extended-spectrum beta-lactamase producing *E. coli*.

Significance: Although the use of FQ as a feed additives has been prohibited in Korea, the prevalence of FQ resistance and PMQR genes has increased considerably in swine. The increased FQ resistance may be, in part, due to the increased use of FQ for self-treatment and therapeutic purposes. Therefore, prudent use of FQ in animal farms is warranted to reduce the evolution of FQ-resistant bacteria in the animal industry.

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P1-214 Low Temperature Inactivation Kinetics to Determine Bacteriophage Shelf-life Stability

JOYJIT SAHA, Pushpinder Kaur Litt and Divya Jaroni
Oklahoma State University, Stillwater, OK

❖ ***Developing Scientist Competitor***

Introduction: Bacteriophages, clinically useful and safe in both human and animals have garnered much attention as antimicrobials in food applications. However, efficacy of antimicrobials in the cold distribution chain for foods largely depends on stability at low temperature range. Retaining activity at low temperatures, for longer periods of time could render bacteriophages as cold-stable biocontrol agents. It is therefore important to understand their cold temperature inactivation kinetics at low and ultra-low temperature.

Purpose: Determine cold temperature inactivation kinetics of bacteriophages specific for non-O157 Shiga-toxigenic *Escherichia coli* (STEC).

Methods: Previously isolated bacteriophages, specific for non-O157 STEC (O26, O45, O103, O111, O121, O145), were used. Phages were exposed to low temperatures of 4, -20, and -80°C for 90 days in sealed-tubes. Phage population was enumerated on days 0, 1, 30, 60, and 90, using double-agar-layer technique. First-order model, commonly used to understand temperature-degradation kinetics, was fitted through regression module, using SigmaPlot13™ (Systat Software, US). The model was evaluated using regression coefficient (r^2), mean square error (MSE), and standard error of means (SEM). Several kinetic parameters: order of reaction (n), half-life ($t_{1/2}$), and inactivation-time (D-value), for low-temperature inactivation were calculated.

Results: The r^2 values obtained from first-order model ranged from 0.82-0.98 for all phages. Irrespective of the host, all phages remained stable at storage temperatures for 90 days, except O103-phage which lost activity on day-90 at -20 and -80°C. The inactivation rate constant (k) for all the phages at 4°C ranged from 5.0×10^{-4} to 1.10×10^{-3} per day. However, it increased slightly at -20°C ($1.80\text{x}10^{-3}$-3.03×10^{-2}) and -80°C (1.47×10^{-3}-3.37×10^{-2}). Predicted D-values for all phages at 4, -20 and -80°C, ranged from 2.03-12.61, 0.20-3.50, and 0.18-2.52 years, respectively. The high predicted D-values indicate that bacteriophages are stable at low and ultra-low temperatures.

Significance: Tested bacteriophages exhibited cold-storage stability, enabling them to be used as biopreservatives in cold chains.

P1-215 Bacteriophage Fitness Indicated by Modeled Adsorption Efficacy

JOYJIT SAHA, Pushpinder Kaur Litt and Divya Jaroni
Oklahoma State University, Stillwater, OK

❖ ***Developing Scientist Competitor***

Introduction: Biofilm-forming shiga-toxin-producing *Escherichia coli*(STEC) are an emerging problem in the food industry, rendering traditional antimicrobials ineffective. Bacteriophages, natural bacterial predators, could be effective biocontrol agents against STEC. Their mode of action involves adsorption through host bacterial cell-membrane matrix. Therefore, understanding the nature of phage adsorption, using reliable predictive models, is crucial for their applicability in food system.

Purpose: To predict bacteriophage fitness through adsorption efficiency

Methods: Isolated bacteriophages, specific for non-O157 STEC (O26, O45, O103, O111, O121, O145), were used. For adsorption experiment, overnight cultures of non-O157 STEC were centrifuged and re-suspended in buffer along with respective phage, at 0.1 multiplicity-of-infection. The mixture was incubated at 37°C and sampled for 80 mins with 20 mins interval, followed by serial-dilution and filtration. Filtered samples were assayed to determine residual phage population using the double-agar-layer technique. Adsorption rate (k) and efficiency (€) was determined through adsorption-kinetics model using SigmaPlot13™(Systat Software, US). Adsorption-equilibrium time was also determined; hypothetically assuming that adsorption process reached an equilibrium. Diffusion constant (D) was determined using standard equation.

Results: The r^2 values (0.93-0.99) suggested that chosen adsorption-kinetics model best described the adsorption data for all phages. The k-values varied from $1.46\ \text{x}10^{-5}$ to $7.41\ \text{x}10^{-5}$ PFU/ml/s indicating that phages had varied adsorption rates with susceptible host. All the phages had high adsorption rates, with the highest € observed at 74%. Adsorption-equilibrium time was determined to be 40 mins for all phages. D-values for all phages varied from $1.18\ \text{x}10^{-1}$ to $2.32\ \text{x}10^{-2}$cc/s. These results indicate that bacteriophages with high adsorption-rate, coupled with high diffusion constant, can be regarded as "fit" to disrupt STEC cell membranes.

Significance: Bacteriophage fitness and equilibrium time could help select potential phages for the development of application protocols in food-systems.

P1-216 Effect of pH on the Fate of Novel Bacteriophages Targeting Non-O157 Shiga-toxigenic *Escherichia coli*

JOYJIT SAHA, Pushpinder Kaur Litt and Divya Jaroni
Oklahoma State University, Stillwater, OK

❖ ***Developing Scientist Competitor***

Introduction: Shiga-toxin-producing *Escherichia coli*(STEC) are responsible for multiple foodborne illness outbreaks. It is therefore crucial to devise effective control strategies. Bacteriophages are advantageous over traditional antimicrobials by virtue of their high-specificity against target bacteria. The FDA-approval of bacteriophages, in ready-to-eat meat and poultry products, has paved way for development of phage-based antimicrobials. However, designing their application method as bio-preservatives in the food systems requires knowledge of critical points such as pH-inactivation parameters. Modeling pH-inactivation of STEC-specific bacteriophages would enable their predicted survival in acidic and alkaline environments.

Purpose: Evaluation of inactivation models for selected bacteriophages and analysis of optimum pH treatment parameters.

Methods: Bacteriophages isolated from cattle farms in Oklahoma, exhibiting inhibition towards non-O157 STEC (O26, O45, O103, O111, O121, and O145) were used. The pH-inactivation of phages was tested over a pH range: 1-11. Surviving population was enumerated at 0, 1, 2, 4, 6, 12 and 24 hours, using double-agar-layer technique. Applicability of first order and Weibull distribution models was evaluated to understand degradation kinetics through regression module, using SigmaPlot13™ (Systat Software, US). Models were evaluated and compared on the basis of regression coefficient (r^2), mean square error (MSE), and standard error of means (SEM).

Results: The results confirmed that Weibull model provided a perfect description of the data. The r^2values ranged from 0.82-0.99 for all phages. Predicted inactivation reliable time (Tr), analogous to D-value, of tested phages ranged from 3.17 h at pH 1 to 1,214,889 h (138 years) at pH 5. Optimum activity of all phages ranged from pH 5.07-5.93 except J-4 (O121) showing optimum activity only at pH 7.13. Positive skewness indicated that bacteriophages showed more stability at slightly acidic pH (5.07-5.90) than near neutral and alkaline pH.

Significance: pH-inactivation model parameters reveal that isolated bacteriophages could be successfully used as biopreservatives in acidic and low alkaline foods.

P1-217 Efficacy of Chlorinated Nanobubble Solutions to Control Shiga Toxin-producing *Escherichia coli*, *Salmonella* spp., and Non-pathogenic *Escherichia coli* Surrogates in Chilled Solutions

AMANDA WILDER[1], Austin McDaniel[2], Randall Phebus[1] and Christopher Vahl[3]

[1]Kansas State University, Manhattan, KS, [2]Kansas State University, Food Science Institute, Manhattan, KS, [3]Kansas State University, Department of Statistics, Manhattan, KS

Introduction: Antimicrobial interventions are effective to reduce pathogen risks on processed produce, meat, and poultry products. Chlorinated solutions have been widely studied in produce and poultry wash waters, but have not been evaluated with inclusion of nanobubble technology. Interest in nanobubble technology has increased due to its proposed surfactant properties; however, it is undetermined whether this technology aids in effectiveness of chlorine-based solutions in food applications.

Purpose: Benchtop studies were conducted to evaluate the efficacy of chlorinated nanobubble waters against Shiga toxin-producing *Escherichia coli* O26, O45, O103, O111, O121, O145, and O157:H7 (STEC-7), *Salmonella* spp., and USDA-approved non-pathogenic STEC surrogates in pure culture to characterize the lethality contributions of pH (5 or 7), temperature (1.6 or 5.6°C), free available chlorine (FAC) level, inclusion of nanobubbles, or a combination thereof.

Methods: Chlorinated waters (0 to 11.94±0.97 ppm FAC) were manufactured with and without inclusion of nanobubbles, and with and without CO_2 gas addition to adjust pH. Pure culture cocktails of STEC-7, *Salmonella* spp., and surrogates were added to chlorinated water treatments for 60 seconds, subsequently neutralized, and surviving population levels quantified.

Results: Surrogate organisms demonstrated greater resistance ($P \leq 0.05$) across all combinations of chlorinated solution treatments (3.4-5.5 log CFU/mL reductions) than pathogens tested; thus, proving to be an appropriate surrogate for STEC and *Salmonella* for commercial in-plant studies. Higher reductions ($P \leq 0.05$) were observed across all target organisms at high (11.94±0.97 ppm) FAC levels. STEC-7 and *Salmonella* population reductions were also notably reduced (3.3-7.1 log CFU/mL) by the chlorinated nanobubble waters. No definitive impacts of temperature, nanobubble inclusion, or acidic pH were observed in pure solutions.

Significance: Determining the impact of nanobubble inclusion in chlorinated solutions will allow for increased knowledge of the technology and its contributions to food safety applications.

P1-218 Biocontrol of Shiga-toxigenic *Escherichia coli* Using Lytic Phages on Mung Beans and Germinated Sprouts

YIRAN DING[1], Yan Dong Niu[2], Kim Stanford[3], Richard Holley[1], Tim McAllister[4] and Claudia Narvaez[1]

[1]University of Manitoba, Winnipeg, MB, Canada, [2]Alberta Agriculture and Forestry, Lethbridge, AB, Canada, [3]Alberta Agriculture, Lethbridge, AB, Canada, [4]Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

Introduction: The big seven Shiga- toxigenic *Escherichia coli* (STEC) strains, which includes serogroups O26, O45, O111, O103, O121, O145, and O157, are recognized as a major cause of enteric disease worldwide.

Purpose: This study evaluated the effectiveness of seven bacteriophages, isolated from beef cattle, to reduce *E. coli* serotypes O26, O45, O103, O111, O121, O145, and O157 on mung beans (MB) and sprouts.

Methods: Sixty samples of MB (30g/treatment) were immersed in a cocktail of the top seven STEC strains to achieve a concentration of (10^4CFU/g). MB were treated with chlorinated water (1000 ppm), STEC lytic phage cocktail (10^7 - 10^9 log PFU/ml) for 1 h, or a combination of both treatments. Beans were then stored for at least 72 h before plating and germination. No treatment was applied during germination. STEC survival in both MB and MB sprouts were assessed using total plate count method on MacConkey and Rainbow agars. Survival of different serogroups was evaluated using immunomagnetic separation and latex agglutination.

Results: The phage cocktail showed lytic activity against the seven STEC serogroups to different degrees. There was an interaction among treatment and serogroup (P<0.0001). Four of seven serogroups exhibited more than three log reductions with phage treatment of mung beans (O45, O103, O145, O157). As expected, phage lytic activity improved when combined with chlorinated water. On germinated sprouts, all serogroups survived; only *E.coli* O111 had more than five log reductions after phage treatment.

Significance: STEC phages were able to reduce the number of STEC in mung beans. These results are promising for future application of phages, in combination with other hurdles, to reduce STEC on sprouting seeds.

P1-219 Detection Extraction and Evaluation of Phage Depolymerase Enzyme against Shiga-toxigenic *Escherichia coli* Biofilms

BEATA MACKENROTH, Pushpinder Kaur Litt and Divya Jaroni

Oklahoma State University, Stillwater, OK

✦ ***Undergraduate Student Award Competitor***

Introduction: Shiga-toxigenic *Escherichia coli*(STEC) is a major group of foodborne pathogens that can produce strong biofilms on foods, and food contact surfaces. These biofilms can be difficult to remove using conventional sanitizers, but could be disrupted using bacteriophage, targeting specific bacteria. These phages produce depolymerase enzymes, to degrade STEC biofilms, allowing the phage to kill the host bacterium.

Purpose: To evaluate biofilm inhibiting activity of depolymerase enzyme extracted from STEC specific phage.

Methods: Previously sequenced phages, isolated in the lab, were tested for the presence of depolymerase-enzyme-coding gene in STEC-O45 using homologous sequence alignment tool (BLAST), PCR and gel electrophoresis. Crude depolymerase-enzyme was extracted using phage-lysate, centrifugation, dialysis, PEG concentration, and ultracentrifugation. Crude extract was then tested for inhibition against STEC-O45, using spot-on-lawn assay. Additionally, its biofilm disrupting capabilities were examined by allowing STEC-O45 (7 $\log_{10}$ CFU/ml) to form biofilms in micro-titer plates for 24 hrs. Plates were then treated with phage, phage-depolymerase or PBS-control for 16 hrs and stained with crystal-violet. Biofilm disruption was measured as a change in absorbance (A_{595}) and was visually analyzed with Scanning Electron Microscopy (SEM). Surviving bacterial population was also enumerated on tryptic soy agar.

Results: Sequences homologous to phage-enzyme were used to design primers. The presence of enzyme-encoding sequence in the phage was confirmed by PCR-positive band at ~800bp. Crude phage-enzyme extract showed lytic activity against STEC-O45 on spot-on-lawn assay. Phage and phage-enzyme treated wells showed reduction in absorbance (0.705 to 0.918) of STEC biofilms, compared to control. A reduction of 1.0-1.1 logs in

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STEC population was observed with phage and phage-depolymerase treatments. SEM images showed an extracellular matrix surrounding the colonies in PBS-control. However, phage and phage-depolymerase treated cells showed rough surfaces with filament-like cell envelopes, indicating cellular damage.

Significance: Bacteriophage and phage-depolymerase can be applied as bio-control agents in the food industry to control STEC biofilms.

P1-220 A Survey of Antimicrobial Resistance among Dairy Cattle in Kosovo

SULAIMAN ALJASIR[1], Jeffrey Chandler[2], Afrim Hamidi[3], Driton Sylejmani[3], Baolin Wang[1], Katherine Schwam[1] and Bledar Bisha[1]

[1]University of Wyoming, Laramie, WY, [2]U.S. Department of Agriculture-APHIS-WS-NWRC, Fort Collins, CO, [3]University of Pristina, Pristina, Kosovo

❖ ***Developing Scientist Competitor***

Introduction: The overuse and abuse of antimicrobials during food production is a threat to public health. Antibiotics are routinely used in dairy cattle to treat mastitis, respiratory infections and other conditions and may ultimately play a role in the emergence and dissemination of antimicrobial resistance in the food supply.

Purpose: The purpose of this study was to conduct a survey of antimicrobial resistance in dairy cattle in Kosovo through isolation and characterization of the indicator microorganisms *Escherichia coli* and *Enterococcus* spp.

Methods: A cross-sectional study was conducted in the summer of 2014 on dairy farms representing the majority of dairy production regions of Kosovo. Fresh fecal samples were collected from 10 cattle in each of the 52 farms included in the study. Individual samples were mixed to form composite fecal samples (500 g) for each farm (n = 52). Isolation was performed on McConkey agar (*E. coli*) and Enterococcosel agar (*Enterococcus* spp.) or media supplemented with ciprofloxacin, erythromycin, or cefoxitin. Isolate confirmation was carried out by MALDI Biotyping. Antimicrobial susceptibilities were determined via the disk diffusion method according to CLSI guidelines.

Results: The highest percentage of resistant *E. coli* isolates (n = 165) were to ampicillin (29.7%), followed by cefazolin (26%) and tetracycline (19.4%). Notably, resistance was also high for piperacillin (17.6%), nalidixic acid (17%) and ciprofloxacin (13.3%). Among enterococci (n = 177), *E. faecalis* isolates were primarily resistant to erythromycin (36.5%), rifampin (33.8%), tetracycline (28.4%) and ciprofloxacin (27%). Similar trends were observed for *E. faecium*, with high levels of resistance to rifampin (52%), tetracycline (38%), ciprofloxacin (36%), doxycycline (28%) and erythromycin (20%).

Significance: This is the first survey of antimicrobial resistance in dairy operations of Kosovo, providing a baseline of antimicrobial resistance within this important niche of food production.

P1-221 Extended-spectrum β-Lactamase Producing *Escherichia coli* in Feed, Manure, and Soil from the Poultry Farm Environment

AGNES KILONZO-NTHENGE, Samuel Nahashon and Siqin Liu

Tennessee State University, Nashville, TN

Introduction: Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* is an emerging problem that is associated with antimicrobial use in food-producing animals. It is essential to assess the potential reservoirs of ESBL-producing *Escherichia coli* in agricultural settings.

Purpose: The aim of the current study was to estimate the prevalence of ESBL-producing *Escherichia coli* in feed, feces, and soil from poultry houses and farm compound. In addition, *E. coli* was evaluated for resistance to antimicrobial drugs.

Methods: A total of 150 samples of feed, feces, and soil were collected for analysis. Biochemical identification of the isolates was performed using oxidase and API 20E test methods. *Escherichia coli* isolates, identified at 98% confidence level, were screened for ESBLs by plating on ChromID ESBL agar plates. Cefotaxime (30 µg) and ceftazidime discs (30 µg), with or without clavulanate (10 µg), were used to confirm ESBL-producing *E. coli.* The antimicrobial susceptibility was determined using the Bauer and Kirby disk diffusion technique. ESBL-producing isolates were also analyzed for the presence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes by PCR.

Results: ESBL-producing *E. coli* isolates were detected in 4.3%, 15.2%, and 80.5% in feed, soil, and poultry feces, respectively. About two-third of the isolates were found to be susceptible to all the antimicrobials used in this study; 31.6% (36/114) of the isolates were resistant to one or more of the antimicrobials tested. Multidrug resistance to three or more antimicrobials was observed in 21.9% (*n*=25) of the *E. coli. Escherichia coli* isolates were resistant to critically important human antibiotics, like erythromycin (99.1%; *n*=113), tetracycline (84. 2%; *n*= 96), amikacin (14.9%; *n*=12), and ciprofloxacin (3.5%; *n*=4). The bla_{CTX-M}, bla_{SHV}, and bla_{TEM} β-lactamase genes were also detected in ESBL-producing *E. coli*.

Significance: This study provides evidence that poultry farms could possibly be a potential source for ESBL-producing *Escherichia coli*.

P1-222 Antibacterial Activity of D-Tryptophan against *Salmonella enterica* and *Escherichia coli* O157:H7 under Osmotic Stress and Its Application to Oyster Preservation

JIAN CHEN, Shuso Kawamura and Shigenobu Koseki

Hokkaido University, Sapporo, Japan

❖ ***Developing Scientist Competitor***

Introduction: The adaptive response of bacteria to osmotic stress includes accumulation of compatible solutes, such as glycine-betaine, to maintain homeostasis. This survival strategy has protective effects for bacterial cells and makes it difficult to control bacteria in low water activity (a_w) foods. In our previous study, D-tryptophan, an incompatible solute that is structurally similar to compatible solutes, had adverse effects on the growth of foodborne bacteria under high salt conditions.

Purpose: This study evaluatee the inhibitory effects of D-tryptophan on *Escherichia coli* O157:H7 and *Salmonella enterica* under various mixed NaCl and D-tryptophan stress conditions and, subsequently, test its antibacterial efficacy against *E. coli* O157:H7 in freshly shucked oysters.

Methods: We examined the antibacterial effect of D-tryptophan on *E. coli* O157:H7 and *S. enterica* in liquid media with various NaCl concentrations. A model identifying the boundary between growth/no growth of the bacteria as a function of NaCl and D-tryptophan concentration was developed by logistic regression with R statistical software. In addition, freshly shucked oysters were artificially contaminated with *E. coli* O157:H7 in artificial seawater (3.5% NaCl) with or without 40 mM D-tryptophan. Bacterial survival was enumerated using plate counts.

Results: Growth/no growth boundary models were successfully developed for both pathogens. *Salmonella enterica* and *E. coli* O157:H7 growth was affected at NaCl concentrations >3% and 4%, respectively, in the presence of 20 mM D-tryptophan; but higher concentrations of both substrates were needed for efficient growth inhibition. In addition, adding D-tryptophan significantly inhibited *E. coli* O157:H7 growth in freshly shucked oysters.

Significance: D-tryptophan may act as a novel, natural food preservative to control foodborne bacterial growth, protect low a_w food quality, and extend the shelf-life of processed seafood.

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P1-223 Prevalence of Antibiotic-resistant Enteric *Escherichia coli* Isolated from Fecal Samples of Food Handlers in Qatar

WALID ALALI[1], Nahla Eltai[2] and Marwan Abou-Madi[2]
[1]*Hamad Bin Khalifa University, Doha, Qatar,* [2]*Qatar University, Doha, Qatar*

Introduction: Bacterial resistance to antibiotics is a major clinical and public health problem. The use and misuse of antibiotics, poor infection control practices, inadequate sanitary conditions, and inappropriate food-handling encourage the further spread of antibiotic resistance.

Purpose: The objective of this study is to determine the prevalence of antibiotic resistant (AR) enteric *E. coli* isolated from fecal samples of immigrant food handlers in Qatar. There are currently limited information on antibiotic resistance profiles in commensal enteric organisms from non-clinical human populations in Qatar.

Methods: Food handlers arriving to Qatar for work go through a mandatory medical screening at the Medical Commission. The screening include submitting a fecal sample for pathogen monitoring. A randomly selected subset of the fecal samples (*n*=178) were used for *E. coli* isolation and antibiotic susceptibility testing using E-test Strip™ (BioMerieux, France) against 9 antibiotics (Ampicillin, trimethoprim, sulfamethoxazole, chloramphenicol, ceftriaxone, gentamicin, tetracycline, ciprofloxacin, and amoxicillin-clavulanic acid).

Results: Nearly 29% of the fecal samples (*n*=178) were positive for *E. coli*. Thus far, 36 *E. coli* isolates have been characterized for antibiotic susceptibility. About 53% of these isolates were resistant to at least one antibiotic; whereas 33.3% of the isolates were multi-drug resistant (those resistant to three or more antibiotics). The highest resistance prevalence was to trimethoprim (39%), followed by ampicillin (36.1%), and sulfamethoxazole and tetracycline (33.3%, each). Resistance to ciprofloxacin (16.7%) and ceftriaxone (5.6%) were observed.

Significance: It appears that the study subjects (food handlers that are, in general, healthy individuals), carry an array of multi-drug resistant enteric *E. coli* bacteria, which can pose a significant public health risk to the general population in Qatar. This risk magnitude is dependent on AR *E. coli* dissemination through food contaminated due to inappropriate food-handling.

P1-224 Prevalence and Characterization of Antimicrobial Resistance Patterns of *Campylobacter* Associated with Poultry

MATTHEW BAILEY[1], Rhonda Taylor[1], Jagpinder Brar[1], Estefania Novoa Rama[1] and Manpreet Singh[2]
[1]*Purdue University, West Lafayette, IN,* [2]*University of Georgia, Athens, GA*

❖ ***Developing Scientist Competitor***

Introduction: Foodborne illness due to *Campylobacter* infection is an important safety issue to the poultry industry. Antimicrobial resistant (AMR) *Campylobacter* strains are of particular concern, leading to increased pressure to reduce the use of antimicrobials during animal production.

Purpose: In this study, the efficacy of antibiotic-free (ABF) management practices was assessed by monitoring the prevalence of AMR *Campylobacter* in a commercial broiler processing plant.

Methods: Fecal grab samples, carcass rinses, water samples, equipment swabs, and air samples were collected from a commercial processing plant on 16 different days within a 12-month time frame. *Campylobacter* was isolated by direct plating on Campy-Cefex agar along with simultaneous enrichment in Bolton's broth. Two isolates were collected per positive sample and confirmed by PCR. These isolates were then tested for antimicrobial resistance using commercially prepared broth micro-dilution plates and the National Antimicrobial Resistance Monitoring System (NARMS) protocol.

Results: A total of 312 PCR-confirmed *Campylobacter* isolates were tested for AMR and 7.1% (22 isolates) were resistant to 3 or more antimicrobials. The most common resistance observed was against tetracycline, with a prevalence of 72.8% (227 isolates). This was followed by nalidixic acid resistance at 7.4% (23 isolates), clindamycin resistance at 6.7% (21 isolates), and ciprofloxacin resistance at 6.1% (19 isolates). Resistance to all other antimicrobials tested was under 3.0%, including erythromycin (2.2%).

Significance: These results demonstrate that AMR *Campylobacter* remains present even in plants that process ABF chicken. Of particular concern was the detection of isolates resistant to important drugs for treating severe campylobacteriosis such as erythromycin (7 isolates) and ciprofloxacin (21 isolates). These findings reinforce the importance of interventions to control overall pathogen prevalence and highlight the continuing need for research on management strategies for AR pathogens.

P2-01 Detection of Antibiotic Residues in Poultry Feathers and Claws by Liquid Chromatography Tandem Mass Spectrometry

JAVIERA CORNEJO, Ekaterina Pokrant, Ricardo Riquelme, Constanza Avello, Karina Yevenes, Aldo Maddaleno, Carolina Araya-Jordan and Betty San Martin
Universidad de Chile, Santiago, Chile

Introduction: Antibiotics can remain in products derived from animals after cessation of drug therapies. Byproducts of the avian industry could become a risk in food safety, if antibiotic residues remain in high concentrations after treatment. Some byproducts as feathers and claws, have shown a high affinity for antibiotics, suggesting the persistence of these drugs in nonedible tissue.

Purpose: This study investigated the transfer of antibiotics from two antimicrobial families (tetracycline and chloramphenicol derivatives) to feathers and claws of therapeutically treated broiler chickens.

Methods: Broiler chickens (220, one-day-old) were kept with ad libitum access to water and nonmedicated feed. For each experiment, two experimental groups were formed: group (A) treated with a therapeutic dose, and group (B) not treated. Eighty birds were used for the oxytetracycline (OTC 10%) study in feathers and claws and 80 birds for florfenicol (FF 10%) in feathers and claws. After treatment, concentrations of the analytes were detected and quantified using LC-MS/MS.

Results: FF plus florfenicol-amine mean concentrations in feathers were 4,161 μg kg^{-1} at day five and 116 μg kg^{-1} at day 40 posttreatment. In claws, concentrations of 484 and 83 μg kg^{-1} were detected at day five post-treatment. OTC and 4-epi-oxytetracycline mean concentrations detected in feathers were 5,609 and 190 μg kg^{-1} at day five and 19 posttreatment, respectively. For claws, 299 and 104 μgkg^{-1}were detected at the same sampling points.

Significance: High levels of antibiotics remained for longer periods in the studied byproducts of treated birds. Results demonstrate that these nonedible tissues may represent a risk for the reentry of antimicrobial residues into the food chain.

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P2-02 Determination of Aflatoxin B1 in Vegetable Oils Using Low-temperature Clean-up Combined with Immunomagnetic Solid-phase Extraction

Xi Yu and **HONGSHUN YANG**
National University of Singapore, Singapore, Singapore

Introduction: Aflatoxins are secondary metabolites predominately produced by the fungi groups *Aspergillus flavus* and *Aspergillus parasiticus,* which widely contaminate agriculture produce and are transferred into vegetable oils during manufacturing process. Since the toxicity of aflatoxin B1 (AFB1) has raised concerns and vegetable oils are an important component of our daily diet, the accurate detection of AFB1 in vegetable oils is necessary and critical.

Purpose: This stuy developed a highly convenient and sensitive method to detect AFB1 in vegetable oils.

Methods: Antibody functionalized magnetic nanoparticles were synthesized and applied for the immuno magnetic solid phase extraction of AFB1 in vegetable oils after low temperature clean-up. The analyte in the extract was quantified using HPLC hyphenated with a fluorescence detector afterwards.

Results: Antibody functionalized magnetic nanoparticles were successfully synthesized and applied to extract the AFB1 in five kinds of oils (corn, canola, peanuts, soybean, and olive oil). The average diameter of Fe_3O_4 nanoparticles was 11.4±3.5 nm. The saturation magnetic moment of Fe_3O_4 nanoparticles and ammonia group functionalized nanoparticles were 48.9 and 33.1 emu/g, respectively. The calibration curve for quantifying AFB1 was successfully built with R^2value of 0.9957, suggesting acceptable linearity of the method applied. Interestingly, none of the oil samples tested were contaminated with AFB1 in the present study.

Significance: The results suggested that the proposed method is promising for detecting trace amount of AFB1 in vegetable oils.

P2-03 Food Safety Knowledge and Practices of Lebanese Food Handlers in Lebanese Households

HUSSEIN F. HASSAN, Hani Dimassi and Zeina Nakat Karam
Lebanese American University, Beirut, Lebanon

Introduction: Data of reported foodborne illnesses suggest that a significant proportion is attributed to improper food handling at the domestic level.

Purpose: The aim of this study was to assess the level of food safety knowledge and practices among 1,500 Lebanese food handlers in Lebanese households and to identify the association between their knowledge/practices and the sociodemographic characteristics.

Methods: Participants were from different gender, age groups, area of residence, income, marital status, and education. They completed a questionnaire of six questions about their demographic characteristics and 26 questions related to their knowledge and practices in terms of food handling, storage, usage of kitchen facilities, and personal hygiene subgroups. Approval of the Institutional Review Board in the Lebanese American University was obtained prior to approaching the participants.

Results: On average, participants scored 55.6±16.3, 51.3±25.7, 67.4±19.3, and 89.1±16.3%, respectively; whereas, the passing rates (above 50%) were 64.5, 69.9, 90.5 and 99.1%, respectively, on the different subgroups. Gender had a significant (P<0.05) effect on food handling and personal hygiene; age, marital status, and education had a significant (P<0.05) effect on handling, usage of kitchen facilities, and personal hygiene; area of residence had a significant (P<0.05) effect on storage, handling, and usage of kitchen facilities; income had a significant (P<0.05) effect on handling and usage of kitchen facilities. In terms of overall food safety knowledge and practices, the mean score was 63.8±12.6%; passing rate was 86.2%; gender, age, area of residence, education, marital status, and income had a significant (P<0.05) effect. Food safety practices and knowledge scores were significantly (P<0.001) related with a weak to moderate correlation coefficient (R=0.34).

Significance: Our results confirm the need for ongoing educational initiatives to improve food safety knowledge and practices among Lebanese food handlers in Lebanese households.

P2-04 *Lactobacillus rhamnosus* GG Inhibits BID-dependent Apoptosis in Human Hepatocellular Carcinoma Cells Exposed to Patulin

BERNICE KARLTON-SENAYE[1], Rishipal Bansode[1], Priscilla Randolph[1] and Leonard Williams[2]
[1]North Carolina A&T State University Center of Postharvest Technologies (CEPHT), Kannapolis, NC, [2]North Carolina A&T State University-Center of Postharvest Technologies (CEPHT), Kannapolis, NC

Introduction: Patulin, a mycotoxin, which is a major contaminant in apple juices, has contributed immensely to the occurrence of liver diseases. Consumption of apple juice could, over long period of time, become harmful to the health of individuals with pre-existing liver disease. Probiotics are known for their role in patulin removal from aqueous media.

Purpose: The purpose of this study was to determine the effects of a probiotic microorganism on patulin toxicity in hepatocellular carcinoma (HepG2) cells and established the protective effect of *Lactobacillus rhamnosus*as mediated by induction of BID in response to patulin toxicity.

Methods: HepG2 cells were seeded in 24 well plates (10^5 cells/well) in EMEM containing 10% fetal bovine serum for 24 h to ensure cell adherence. Cells were exposed to patulin at 0, 1, 2.5, 5, 7.5, and 10 µM for 24 h followed by treatment with Lactobacillus rhamnosus GG (LGG) for 24 h. Total protein normalization and western blot were conducted to determine the expression of PUMA and BID.

Results: After 24 h of patulin exposure, followed by 24 h of treatment with *Lactobacillus rhamnosus* (LGG), cells proliferation decreased with increasing patulin exposure in samples without LGG pretreatment; whereas, with increasing concentration of patulin, cells were relatively rescued in LGG treated samples. It was further observed that pretreatment of LGG with polysaccharide gums led to a decline in cell proliferation with increasing patulin exposure. Compare to the control, the expression of PUMA increased slightly, by 7%, at 10µM patulin exposure in treatment. However, the expression of BID decreased by 26% in treatment compared to the control showing the protective effect of LGG.

Significance: Our findings suggest that LGG could potentially function as a therapeutic agent to reverse the damaging effect of patulin on the liver of individuals with pre-existing liver disease.

P2-05 Assessment of Aflatoxin M_1 and Heavy Metals in Human Milk Samples from Pakistan

AMIR ISMAIL[1], Sarah Khan[1], Muhammad Riaz[1], Saeed Akhtar[1] and Yun Yun Gong[2]
[1]*Bahauddin Zakariya University, Multan, Pakistan,* [2]*Leeds University, Leeds, United Kingdom*

❖ ***Developing Scientist Competitor***

Introduction: Mother's milk is the most vital food item for the entire life of a human as the infants completely depend on mother's milk for the initial months of their life. Toxicity through mother's milk, at this stage, may affect their whole life.

Purpose: The present study was aimed to assess the level of some major toxicants in mother's milk including aflatoxin M_1 (AFM_1), heavy metals, lead (Pb), and cadmium (Cd).

Methods: To present a clear picture, mother milk samples (*n*=80) were collected on the basis of economic classes i.e. poor, middle class and rich. AFM_1level was assessed by using ELISA kits, while flame atomic absorption spectrophotometer was used for the quantification of heavy metals.

Results: AFM_1 level was found in the range of 0.0167 to 0.0303 µg/liter. Twenty-five percent of mother's milk samples were found exceeding the maximum permissible limit for AFM_1 proposed by WHO (0.025 µg/liter). The ranges of Pb and Cd in mother's milk samples were 0.09 to 0.43 µg/ml and 0.022 to 0.094 µg/ml, respectively. All of the mother's milk samples were found exceeding the WHO maximum permissible limits for both Pb (5 µg/liter) and Cd (1 µg/liter). The order of $AFM_1$and heavy metals on socioeconomic grounds was poor > middle class > rich.

Significance: Findings of the study indicated that the food safety situation of mothers in Pakistan. Strict measures are needed by the regulatory agencies to control the level of aflatoxins and heavy metals in the population of Pakistan.

P2-06 Assessment of Selected Metal Concentrations in Shelf-stable Commercial Apple Juices and Fresh Apple Ciders in Michigan

LOAN CAO and Leslie Bourquin
Michigan State University, East Lansing, MI

❖ ***Developing Scientist Competitor***

Introduction: Concern about the presence fo arsenic and other metals in fruit juices has been heightened due to their potential toxicity. Prior surveys of metal concentrations in apple juices have not considered their points of origin (i.e., domestic versus foreign). Therefore, it is unclear if differences exist between shelf stable apple juices (which, in the United States, would largely be produced using imported apple juice concentrate) and fresh juices produced from domestic apples.

Purpose: This research was conducted to assess the concentrations of different metals, particularly arsenic (As), aluminum (Al), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb) and manganese (Mn), in shelf-stable apple juices and fresh apple ciders obtained at retail establishments and cider mills throughout Michigan.

Methods: Samples of shelf-stable apple juice and fresh apple cider, obtained in the fall of 2015 and 2016, were analyzed for metal concentrations by inductively coupled plasma mass spectrometry (ICP-MS).

Results: Averaged across both years, As was detected in quantifiable concentrations (> one µg/liter) in 78% of juice samples, but only 16% of cider samples. Among those samples containing quantifiable As and Pb, the total As and Pb concentrations in apple juice and cider were similar. Cu and Mn was detected in all samples, and their mean concentrations did not differ in apple juice and cider tested in 2016. Al and Cr levels were not significantly different among juice categories, but the percentage of samples containing these metals was higher in apple juices.

Significance: The As and Pb concentrations detected in this study were consistently lower than current advisory levels indicated by the FDA. Fresh apple ciders manufactured using local apples had significantly lower percentages of samples that contained selected metals compared to shelf-stable apple juices, which are largely manufactured using imported juice concentrate.

P2-07 Detection of Nickel, Copper and Lead in Food Using Portable XRF

CONNOR SULLIVAN[1], Pradeep Kurup[1] and Andre Senecal[2]
[1]*University of Massachusetts Lowell, Lowell, MA,* [2]*U.S. Army NSRDEC, Natick, MA*

Introduction: Heavy metal contamination of food is a significant health concern across the world, particularly in developing nations. To allow for testing in regions where traditional laboratory analysis is expensive, time consuming or unavailable, a simple, inexpensive, and portable method is needed for the rapid onsite analysis of food to prevent chronic and acute heavy metal poisoning.

Purpose: The purpose of this research was to evaluate a portable X-ray fluorescence (XRF) analyzer for the analysis of nickel, copper, and lead in food, and to develop simple sample preparation procedures and test methodologies that yield reliable results.

Methods: Samples were prepared using two methods. First, samples of lettuce, strawberry, and ground chicken were homogenized in a stomacher and spiked with known concentrations of the analytes. Using this procedure, concentrations of 0 to 90 ppm were tested. In the second procedure the samples were stomached, oven dried at 90°C and then crushed. The second procedure was used to concentrate the analyte to increase sensitivity.

Results: Lettuce, strawberry, and chicken samples prepared by stomaching exhibited detection limits of 20 ppm for nickel and copper and five ppm for lead. Regression plots of predicted concentration versus actual concentration had R^2 values ranging from 0.9449 to 0.9982. The samples prepared by drying and crushing had detection limits for lead of 0.5, 1, and 1 ppm for lettuce, strawberry, and chicken, respectively.

Significance: The results demonstrated that the XRF has the potential to predict heavy metal concentrations in the low ppm level, by stomaching. Additionally, drying and crushing of samples facilitates the detection of high ppb heavy metal concentrations in media with high water content.

P2-08 Development of a Competitive ELISA Method for the Detection and Characterization of Gluten in Fermented and Hydrolyzed Food Products

RAKHI PANDA and Eric Garber
U.S. Food and Drug Administration, College Park, MD

Introduction: **Methods** are lacking for detecting and quantifying gluten in foods subjected to fermentation and other forms of processing that result in hydrolysis. The few commercially available methods are not able to distinguish between different hydrolytic patterns; a severe limitation that makes accurate quantification impossible.

Purpose: To develop a competitive ELISA for the detection and characterization of gluten in fermented and hydrolyzed foods.

Methods: A novel competitive ELISA was developed utilizing five gluten-specific antibodies (G12, R5, 2D4, MIoBS, and Skerritt) employed in nine established commercial ELISA test kits that are routinely used for gluten detection. The assay was used to evaluate 20 wheat beers, 20 barley beers, 5 barley beers processed to reduce gluten, 15 soy sauces, 6 teriyaki sauces, 6 Worcestershire sauces, 5 vinegars, and 8 sourdough breads.

Results: The developed ELISA simultaneously measures gliadin, deamidated gliadin, and glutenin specific epitopes present in gluten. Standard curves were generated with a dynamic range of 2.5 to 100 ppm gluten. Cluster analysis of the data generated by the antibodies in the novel competitive ELISA was able to distinguish between the different categories of fermented and hydrolyzed foods based on their ELISA response profiles. Wheat beers typically showed a high reactivity to the G12 and Skerritt antibodies compared to the R5, whereas sourdough breads produced equal responses to the G12 and R5 antibodies and a comparatively higher response to the Skerritt antibody. Although some of the soy-based sauces showed non-specific responses with multiple R5 and G12 antibodies, their overall profile was distinguishable from the other categories of fermented foods.

Significance: This novel gluten-based competitive ELISA provides insight into the specificity of the fermentation process and the extent of hydrolysis. It has the potential to aid in the selection of appropriate hydrolytic calibration standards, leading to a more accurate estimate of gluten concentration.

P2-09 Detection of Gluten in a Barley-Malt Beer Produced with and without a Prolyl Endopeptidase Enzyme

Magdalena Naziemiec[1], Wanying Cao[1], Liyun Zhang[1], Nicholas Smith[2], Matthew Arbuckle[2], Arnoldo Lopez-Hernandez[2], Lanlan Yin[3], Katherine Fiedler[3], **BINAIFER BEDFORD**[4] and Lauren Jackson[4]

[1]Illinois Institute of Technology, Chicago, IL, [2]University of Wisconsin, Madison, WI, [3]U.S. Food and Drug Administration, College Park, MD, [4]U.S. Food and Drug Administration, Bedford Park, IL

Introduction: Consumers with celiac disease must avoid gluten to maintain good health. Unfortunately, detection of gluten in hydrolyzed and fermented foods poses an analytical challenge.

Purpose: This study examined how the brewing process and the addition of a prolyl endopeptidase enzyme (Brewers Clarex®) affected gluten detection in beer brewed with barley malt using gluten-specific ELISA and lateral flow devices (LFDs).

Methods: Beer (55 L) was brewed from barley malt in a pilot plant, and Clarex®was added at varying dosages [0 (control), 0.017, 0.034, 0.068 ml/liter] to equal volumes (13 L) of beer before fermentation. Beer samples were collected until 8.5 weeks fermentation/aging. Five ELISA tests (Ridascreen Gliadin sandwich, Ridascreen Gliadin Competitive, Aller-Tek Gluten, Morinaga Wheat/Gluten, AgraQuant G12) and four LFD kits (AgraStrip G12, ELISA Systems sandwich, ELISA Systems competitive, Morinaga Gluten/Gliadin IIR) were used to detect gluten in samples. Pilot plant trials and analytical tests were completed in triplicate.

Results: Gluten concentrations in unfermented beer ranged from 96 to 191 ppm gluten across three trials as measured with the AgraQuant G12 ELISA [LOQ, 4 ppm]. After 8.5 weeks fermentation/aging, control beer had <LOQ 10 ppm gluten, while the Clarex®-treated beer (0.017, 0.034, 0.068 ml/liter) had 4 to 21 ppm gluten, <LOQ 35 ppm gluten, and <LOQ 23 ppm gluten, respectively (≤6% CV for all samples). Control beer fermented/aged for 8.5 weeks and subjected to a filtration treatment had up to seven ppm gluten, while comparable Clarex®-treated beer had gluten levels <LOQ of the AgraQuant ELISA. While the AgraStrip LFD tests gave positive results for all unfiltered beer samples, only the control treatment gave positive results for filtered beers.

Significance: Variability exists when determining gluten content in hydrolyzed and fermented foods. Complementary methods, such as mass spectrometry, are needed to detect gluten in fermented and hydrolyzed foods.

P2-10 Development of a Rapid and Nontoxic Procedure for Extraction and Detection of Gluten from Processed Foods

LeAnna Willison, Henry Grise, Ken Roux and **JASON ROBOTHAM**

BioFront Technologies, Tallahassee, FL

Introduction: Gluten is an abundant seed storage protein found in wheat, barely, oat, and rye. Sensitivity to gluten affects 6% of the United States population and includes individuals affected by celiac disease (CD), wheat allergy, and nonceliac gluten sensitivity (NCGS). These individuals experience a range of symptoms upon ingestion of wheat and related cereals.

Purpose: This study was conducted to: i) develop a commercial, monoclonal antibody-based, direct sandwich enzyme-linked immunosorbent assay (ELISA) kit for the detection of gluten in complex food matrices; and ii) design an efficient extraction regime that eliminates hazardous waste issues associated with existing kits.

Methods: A new set of gluten-specific monoclonal antibodies, clones 13F6 and 14F11, were used to develop the MonoTrace Gluten ELISA. For maximum gluten extraction from various commercial and in-house incurred samples, extraction conditions were optimized to one hour at 60°C using a novel gluten extraction buffer (MGEB). Recovery of gluten using the MGEB (60% ethanol, sodium metabisulfite, and glutathione) was compared to the patented Mendez extraction cocktail, which contains a more toxic reducing agent, b-mercaptoethanol (BME), and guanidine hydrochloride (GuHCL).

Results: The newly developed gluten ELISA kit reduces the overall extraction time of gluten-containing products to a single step performed in one hour. Additionally, the requirement for toxic chemicals was eliminated, which negated the need for expensive hazardous waste disposal. Among the 47 tested commercial samples, with and without declared gluten, no false positive or negative results were obtained by the assay. This monoclonal antibody-based gluten sandwich ELISA demonstrated better than or equal recovery in 16 of 27 gluten positive samples, compared to the Mendez extraction method.

Significance: The new gluten ELISA from BioFront Technologies represents a sensitive and robust assay for detecting gluten in a wide variety of complex food systems, while utilizing a novel nontoxic extraction buffer.

P2-11 Review of Recent Advances in the Use of Proficiency Test Data from Fapas

Mark Sykes[1], **JASON ROBOTHAM**[2] and Craig Eaton[1]

[1]Fera Science, Sand Hutton, York, United Kingdom, [2]BioFront Technologies, Tallahassee, FL

Introduction: Proficiency testing (PT) is an essential part of a laboratory's quality assurance processes, whether or not that laboratory is accredited for the analysis in question. For FSMA, PT programs must meet ISO/IEC laboratory accreditation requirements for critical testing requirements in food, pet food and animal feed. Laboratories will use their PT data in charting their performance over time. However, PT data has uses beyond the immediate needs of the participants.

Purpose: PT studies can be used to provide method performance data without recourse to costly collaborative trials. This presentation demonstrates the uses of PT data through examples generated from Fapas proficiency testing services. The examples derive from a variety of studies as well as new reference material products in the Fapas portfolio.

Methods: Data were obtained through proficiency tests accredited to ISO 17043. Data were analysed according to the requirements of the same standard and in keeping with ISO Guide 35.

Results: Critical method parameters including potential matrix interference were derived from PT data where sufficient method information was captured by the PT provider. The PT data further generated reference values and measurement uncertainty values for reference materials.

Significance: All of these additional uses of PT data help demonstrate competency to deliver reliable and accurate results to the analytical community and is especially important in the analysis of chemical contaminants or food allergens. Although yet to be promulgated, FSMA Section 202 calls for laboratory accreditation, so PT data, for single and multilaboratory, should be an essential tool to achieve model laboratory standards.

P2-12 Characterization of the Monotrace Gluten Sandwich ELISA, a Specific and Sensitive Assay for the Detection of Gluten within Processed Foods and Unprocessed Ingredients

HENRY GRISE, LeAnna Willison, Ken Roux and Jason Robotham

BioFront Technologies, Tallahassee, FL

Introduction: There is a growing commercial need for new methods to rapidly and accurately detect gluten in foods for the protection of individuals suffering from celiac disease and nonceliac gluten sensitivity. Gluten is primarily composed of gliadin and glutenin, two abundant seed storage proteins found within wheat, barley and rye.

Purpose: 1) To validate the performance of the MonoTrace™Gluten ELISA using both raw and processed foods. 2) To test this monoclonal antibody-based assay for cross-reactivity (CR) against a large panel of cereals, tree nuts, legumes, seeds, meats and spices.

Methods: The assay limit of detection (LOD) was calculated by testing kit standards in triplicate on three different days. Recovery from various food matrices and ingredients (*n*=18) was analyzed using in-house incurred cornbread samples, gluten-free (GF) commercial products and ingredients, as wells as quality control and reference materials sourced from the Food Analysis Performance Assessment Scheme (FAPAS) division of the Food and Environment Research Agency (FERA). Cross-reactivity was tested using a large panel of GF and nonGF labeled commercial samples from various vendors (*n*=126).

Results: The LOD for this new commercial gluten ELISA was determined statistically to be 0.3 ppm and the range of quantification (ROQ) was determined to be 2 to 100 ppm gluten (1 to 50 ppm gliadin). Recovery of gluten from in-house gluten-incurred cornbread and gluten-spiked commercial food matrices was >75%. No cross-reactivity was observed in any of the tree nuts, legumes, seeds, meats or spice samples tested. Wheat, rye and barley flours were highly CR. The assay also exhibits little to no cross-reactivity to several oat cultivars (*n*=5).

Significance: The BioFront Technologies' gluten ELISA represents a rapid method for detecting and accurately quantifying gluten contamination within raw food ingredients and processed foods. The assay is highly specific, sensitive and has a wide ROQ.

P2-13 Real-time PCR for the Detection of Allergenic Peanut and Tree Nuts

ANNE EISCHEID and Caroline Puente-Lelievre

U.S. Food and Drug Administration, College Park, MD

Introduction: Analysis of food allergens is generally carried out using either ELISA for the detection of proteins from the allergenic food, or PCR for detection of their DNA. Real-time PCR is not only quantitative, but has also been shown to be robust for trace level detection of allergens in a variety of foods and processing conditions.

Purpose: The purpose of this work was to develop real-time PCR assays for the detection of peanut and for the detection of walnut, the primary tree nut allergen.

Methods: Primers and probes for real-time PCR were designed to target the internal transcribed spacer (ITS) regions in the rRNA genes of the target genomes. Primer and probe design used DNA sequence data from numerous types of plants likely to be found in food products, including nontarget tree nuts, legumes, grains, and spices, in order to specifically design the assay not to cross-react with these other plant materials. Real-time PCR was carried out using six to eight target levels corresponding to approximately 0.1 to 10^6 parts per million allergenic food.

Results: Assay performance was evaluated using reaction efficiency, statistical R^2 value, and linear range. Results demonstrate that assay linearity spanned all six to eight orders of magnitude tested. Reaction efficiencies near the ideal of 100% were achieved. In general, there was low cross-reactivity with some of the non-targeted food products tested.

Significance: Food allergies affect approximately 4 to 6% of the U.S. population, and the Food Allergen Labeling and Consumer Protection Act (FALCPA) requires that foods containing any of eight major allergenic foods and food groups be labeled accordingly. This necessitates highly sensitive detection methods. This work was focused on the development of such highly sensitive detection methods for peanut and tree nuts, two of the major food allergens in the United States.

P2-14 A Sensitive and Ara h 2 Specific Competitive ELISA for the Detection of Peanut in Processed Foods

SHYAMALI JAYASENA[1], Steve L. Taylor[2] and Joseph Baumert[1]

[1]University of Nebraska-Lincoln, Lincoln, NE, [2]Food Allergy Research & Resource Program, Department of Food Science & Technology, University of Nebraska, Lincoln, NE

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Introduction: The food industry relies heavily on ELISA-based methods for detecting peanut in food matrices. Although commercial peanut ELISA kits perform quite well with many food matrices, most of them do not perform as well with highly heat processed foods, mostly because they predominantly detect the heat sensitive peanut allergen Ara h 3.

Purpose: The objective of this study was to develop a more robust and sensitive peanut ELISA targeting the heat stable peanut allergen Ara h 2.

Methods: Peanut Ara h 2 was purified from light roast peanut flour using column chromatography techniques. Polyclonal antibodies raised in rabbits against purified Ara h 2 were used to develop a competitive inhibition ELISA targeting Ara h 2. The performance of the developed ELISA in recovering peanut from a solid food matrix (pastry squares) subjected to different processing conditions was compared with that of two commercial peanut ELISA kits.

Results: The developed ELISA was highly sensitive with a detection range of 40 to 400 ng/ml of Ara h 2. The limit of detection and IC_{50} were 27±8 ng/ml and 134±32 ng/ml of Ara h 2, respectively. The Ara h 2 ELISA performed significantly better (P<0.05) than both the Neogen Veratox and Morina-

Poster

ga Peanut ELISA methods in recovering peanut from fried, baked and high pressure processed pastry squares incurred with peanut. With samples subjected to more severe processing, the performance of the developed ELISA was comparable (P>0.05) to the Morinaga ELISA. Among 45 food ingredients tested for cross reactivity with the rabbit antisera, none showed any cross reactivity although minor matrix interference was observed with cloves, cocoa powder, mustard, and pistachios.

Significance: The results indicate that the developed ELISA, targeting a heat stable peanut allergen, shows higher sensitivity than some of the currently available ELISAs in detecting peanut from processed food matrices.

P2-15 Proteomic Identification of Marine Protein Toxin

TAI-YUAN CHEN, Yu-Huai Chang and Deng-Fwu Hwang
National Taiwan Ocean University, Keelung, Taiwan

Introduction: Stonefish have caused a number of human deaths, but the protein profiles of the venom have not been clearly established due to the venom's extreme lability.

Purpose: Performace of feasibility of proteomic approach in identifying fish spine venom.

Methods: To elucidate the toxin components, native polyacrylamide gel electrophoresis (Native-PAGE), sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional electrophoresis (2-DE) were employed in this study to establish the protein pattern profiles of crude venom from *Synanceia verrucosa* in Taiwan. After in-gel digestion, a matrix assisted laser desorption/ionization-quadrupole time of flight mass spectrometer (MALDI-Q-TOF) was used to identify protein samples. MALDI-Q-TOF can analyze PMF and further provide product ion spectra (MS/MS ion search) for protein identification.

Results: The venom estimated by Native-PAGE were 471, 358, 260, and 166 kDa. The predominate protein bands of crude venom were 84 and 75 kDa by SDS-PAGE. The crude venom protein fell in the region with pI values of 7 to 9 and molecular weights of 75 to 90 kDa by 2-DE. Peptide mass fingerprints (PMF) and MS/MS ions originated from MALDI-Q-TOF were used to identify the protein. Our results showed that the complete components of neoverrucotoxin (neoVTX) α-subunit and β-subunit as well as verrucotoxin (VTX) β-subunit were identified from SDS-PAGE and 2-DE patterns.

Significance: This study was to demonstrate the efficacy of current bottom-up proteomic and bioinformatic approaches in identifying proteinaceous venom toxins from *S. verrucosa* spines.

P2-16 Unravelling the Impact of the Bacterial Depsipeptide Cereulide on the Mitochondrial Function of Caco-2 and HepG2 Cells

Marlies Decleer, Sarah De Saeger and **ANDREJA RAJKOVIC**
Ghent University, Ghent, Belgium

Introduction: Cereulide (CER) is a lipophilic cyclododecadepsipeptide produced by *Bacillus cereus*. This toxin is known to induce emetic type of food poisoning, sometimes related with liver failure and even with fatal outcome. In contrast with doses associated with food poisoning, recent prevalence data demonstrated relatively low concentrations of cereulide in rice and pasta dishes. The effects of repeated exposure to low levels of cereulide through food is largely unknown and can lead to subchronic harms.

Purpose: The goal was to provide a multifaceted insight into the impact of a continuous exposure of low doses of cereulide on metabolic responses of Caco-2 and and HepG2 cells as models for intestinal and liver toxicity.

Methods: Caco-2 and HepG2 cells were exposed to food-relevant low concentrations of CER to investigate the effect of a longer exposure. To explore the mechanisms involved in the cytotoxic response and mitochondrial function, the Seahorse Bioscience XFe24 analyzer (Massachusetts, USA) was used in combination with well-established assays for mitochondrial activity (MTT) and changes in protein content (SRB (sulforhodamine B)). The effects of cereulide on the mitochondrial oxygen consumption rate (OCR) were assessed using the Seahorse Bioscience XF Cell Mito Stress Test assay kit. In this assay, modulators of cellular respiration (oligomycin, FCCP, and a mix of rotenone and antimycin A) were serially injected providing insight into different aspects of mitochondrial function. High-resolution mass spectrometry was used to unravel the metabolic profile of CER.

Results: Both MTT and SRB assays showed toxicity on undifferentiated cells at 0.125 ng/ml CER after three days of exposure. The three-day treatment with low concentrations of CER on mitochondrial respiration in intact human intestinal epithelial cells showed perturbations in mitochondrial respiration at a concentration of 0.125 ng/ml.

Significance: These in vitro data suggest that repeated exposure of CER might injure intestinal cells even at relative low doses. Cereulide appear to be more toxic than other cyclodepsipeptide toxins with ionophoretic properties like valinomycin and beauvericin.

P2-17 Antibiotic Contaminations of Locally Formulated Cat Fish Feeds from Southwestern Nigeria

MOMODU OLORUNFEMI[1], Adegboyega Odebode[1], Ifeoluwa Adekoya[2], Patrick Njobeh[3] and Rui Krause[4]
[1]University of Ibadan, Ibadan, Nigeria, [2]University of Johannesburg, Johannesburg, South Africa, [3]University of Johannesburg, Department of Biotechnology and Food Technology, Faculty of Science, Johannesburg, South Africa, [4]Rhodes University, Grahamstown, South Africa

❖ ***Developing Scientist Competitor***

Introduction: In fish farming, antibiotics are added to the fish pool or feed to prevent diseases, maintain hygienic environment and promote growth. However, problems may arise from their toxic residues in animal and their products due to uncontrolled use-age leading to development of resistant microbial strains, an array of diseases and inducement of allergic processes.

Purpose: In this study, the spectrum of antibiotics and their concentration in locally formulated fish feeds were determined to assess their level.

Methods: Ninety-four fish feed samples intended for juvenile *Clarias gariepinus* (Cat fish) were randomly collected from warehouses within Southwestern, Nigeria. Antibiotics in the fish feeds were detected and quantified using a liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results: Six antibiotics were identified namely: monactin, nonactin, dinactin, chloramphenicol, oxytetracycline, and erythromycin. Monactin, nonactin and dinactin produced by members of *Streptomyces* species were detected in 45 (48%), 82 (87%), and 27 (29%) of samples at mean concentrations of 1.5, 0.5, and 8,511.5 µg/kg respectively. Oxytetracycline was the highest recorded antibiotic in the sampled fish feeds with concentration, mean value and frequency of 4,080,000.0 µg/kg, 533,250.0 µg/kg, and 28%. Erythromycin was detected in only three samples however the range and mean concentration was very high at 24,700.0 to 306,000.0 µg/kg, and 188,233.3 µg/kg, respectively. Fish feeds had chloramphenicol at different concentrations in 52 (55%) samples with mean of value 45.4 µg/kg.

Significance: Considering the array and concentrations of antibiotics found in the feeds including those with known toxicities, it could be posited that the consumption of fishes fed with these feeds and their products could pose a significant health risk to consumers particularly oxytetracycline, erythromycin and chloramphenicol.

P2-18 Mitigation of Acrylamide in Foods: An African Perspective

OLUWAFEMI ADEBO[1], Eugenie Kayitesi[1], Janet Adebiyi[1], Sefater Gbashi[1], Judith Phoku[2] and Patrick Njobeh[1]

[1]University of Johannesburg, Department of Biotechnology and Food Technology, Faculty of Science, Johannesburg, South Africa, [2]University of Johannesburg, Water and Health Research Unit, Faculty of Health Science,, Johannesburg, South Africa

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Introduction: Acrylamide (ACR) is a heat generated food toxicant particularly found in carbohydrate-rich foods and considered a possible human carcinogen with neurotoxic properties. Its occurrence in ready-to-eat foods and its toxicological effects on humans is a public concern and constitutes a major challenge to food safety.

Purpose: Particularly in Africa where most carbohydrate-based foods are subjected to varying degrees of thermal processing and consumed as staple diets almost on daily basis, reports on its occurrence is rather too limited. As such, African populations may be exposed to high levels of ACR daily. This study thus provides an overview of the formation, occurrence and health impact of ACR in foods in an African context. It further explored previous studies looking at ACR reduction and mitigation strategies, especially those that may be applicable in the continent.

Methods: An exhaustive review of the literature was done on the formation, health impacts and occurrence of ACR in foods in other regions of the world including Africa. Possible mitigation strategies that can be applied in the continent was also appraised.

Results: Very few studies on ACR in foods have been reported in the continent, despite the different heat related processing of carbohydrate-rich foods that are daily consumed. Possible reduction strategies were also proposed that could help reduce the menace of ACR contamination in African foods. Adequate sensitization of the populace about the prevention of ACR as a food contaminant is however essential to ensure the safety of heat processed carbohydrate-rich foods in the continent.

Significance: This study demonstrated the need for concerted efforts and research towards ACR contamination in Africa.

P2-19 Evaluation of the Microbiological Contamination Levels of Meat Markets Varying by Facility, Processing Temperature, and Market Type

IL-BYEONG KANG[1], Dong-Hyeon Kim[1], Dana Jeong[1], Hong-Seok Kim[1], Young-Ji Kim[1], Hyunsook Kim[2], Joo-Yean Lee[3] and Kun-Ho Seo[1]

[1]Konkuk University, Seoul, South Korea, [2]Hanyang University, Seoul, South Korea, [3]Korea Livestock Products HACCP Accreditation Service, Gyeonggi province, South Korea

Introduction: In meat markets, meat products can become exposed to microbial contamination via contact with workers, utensils, and other processing surfaces.

Purpose: This study was conducted to investigate the relationship between the environmental characteristics of meat markets and the microbiological quality of meat products.

Methods: Ground beef samples (n=80) were purchased from 40 meat markets in Seoul, Korea between July and September, 2015. Samples were subjected to enumeration of mesophilic aerobes (MA) and total coliforms (TC). In addition, samples were evaluated for the presence of common foodborne pathogens found in beef products (*Listeria monocytogenes*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Salmonella* spp.) according to the Korean standard method. Each market was classified according to the following factors: Hazard Analysis Critical Control Point certification (HACCP+), separation of processing areas (SP+), temperature maintenance below 20°C (TEM+), and washing of utensils between processing steps.

Results: There were significantly lower numbers (P<0.05) of TC in TEM+ markets than in TEM- markets, suggesting that temperature control is essential for reducing microbial contamination of meat products. Beef products from wholesale market harbored significantly fewer MA and TC than those sold at department stores and single markets (P<0.05). Notably, however, the factors examined in this study had no significant influence on the prevalence of the four foodborne pathogens tested in beef products.

Significance: The results of this study indicated that temperature control during the meat processing and the fewer handling processes after slaughtering are the key factors that effect microbial contamination levels in retail meat products.

P2-20 Antimicrobial-resistance Patterns of *Salmonella* Isolated from Small-ruminant Carcasses in the United States and Bahamas

KEELYN HANLON, Mark Miller and Mindy Brashears

Texas Tech University, Lubbock, TX

❖ ***Developing Scientist Competitor***

Introduction: Studies on prevalence, characterization and antimicrobial resistance (AMR) patterns of *Salmonella* are common in cattle, swine, and poultry, but scarce information is available for small ruminants. Understanding the current presence of *Salmonella* and any associated AMR is important to improve the safety of retail lamb and goat meat.

Purpose: To determine the presence and AMR patterns of *Salmonella* isolated from goat and lamb carcasses in the United States and Bahamas.

Methods: Carcass surface swabs were collected from 122 goats and 173 lambs at abattoirs in California, New Mexico, Texas and Bahamas over two years. Samples were analyzed using Genedisc® for *Salmonella* detection, with potential positive samples presumptively confirmed by traditional culturing methods and latex agglutination. Isolates were frozen in duplicate with 20% glycerol. Frozen isolates were recovered and subjected to antimicrobial testing using the National Antimicrobial Resistance Monitoring System (NARMS) protocol. AMR was determined using 96-well gram negative plates with predetermined concentrations of a panel of 15 antibiotics, and resistance determined using the Sensititre™ automated antimicrobial susceptibility system.

Results: *Salmonella* was detected on 3.1% of carcass surfaces at preevisceration, 4.8% at post-evisceration, and 2.0% at postintervention (after application of organic acid). Nine *Salmonella* isolates collected at postevisceration, and 12 postintervention isolates were successfully recovered and subjected to AMR testing. According to the predetermined MIC breakpoints tetracycline resistance was found in 10% (n=2) of isolates. Resistance to azithromycin was found in 5% (n=1) of isolates. Intermediate resistance was determined for amoxicillin, ampicillin in 10% (n=2) and chloramphenicol in 5% (n=1) of isolates. None of the isolates expressed multi-drug resistance (MDR).

Significance: This information contributes to the understanding of goat and lamb safety in the retail case. The absence of MDR *Salmonella* from small ruminants is notable, and is preliminary evidence that sheep and goats may not be contributing to MDR development in the food supply system.

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P2-21 *Salmonella* Heidelberg Food Isolates Have Enhanced Attachment to Abiotic Surfaces under Stress Conditions

ALYSSA WEST, Andrea Ray and Haley Oliver
Purdue University, West Lafayette, IN

✦ ***Undergraduate Student Award Competitor***

Introduction: A recent outbreak of salmonellosis from poultry sickened 634 people from March 2013 to July 2014. We hypothesized that the outbreak-associated strains may have enhanced stress tolerance, potentially due to increased biofilm formation, enabling these strains to survive microbial control strategies aimed to reduce pathogens during poultry processing.

Purpose: The purpose of this study was to determine if *Salmonella* Heidelberg outbreak-associated food isolates have greater attachment abilities to abiotic surfaces (an indicator of biofilm forming capacity) than *Salmonella* Newport and Typhimurium food isolates and a *Salmonella* Heidelberg reference strain.

Methods: Attachment was measured using a standard crystal violet assay with modifications. Briefly, 10 *Salmonella* Heidelberg isolates (nine outbreak-associated isolates and reference strain SL476), and eight additional food isolates (four *Salmonella* Typhimurium and four *Salmonella* Newport) were independently grown in TSB to OD_{600} 0.6 to 0.8. Polystyrene 96-well plates were prepared with 1x (nutrient-rich) or 1/20x (nutrient-limited) TSB, inoculated, and incubated at 4°C, 21°C, or 37°C for one, three, or five days. Attached cells were stained with 0.4% crystal violet and indirectly quantified by optical density (OD_{600}). Data were analyzed in SAS (v. 9.4) using ANOVA with significance defined at α=0.05.

Results: When grown in 1/20x TSB at 21°C, six outbreak-associated *Salmonella* Heidelberg isolates had significantly higher attachment on day one, three, and five compared to reference strain SL476 (P<0.0001). Further, two outbreak-associated *Salmonella* Heidelberg isolates had significantly higher attachment than SL476 (P<0.0001) when grown in 1/20x TSB at 4°C. However, outbreak-associated *Salmonella* Heidelberg strains had significantly lower attachment irrespective of time compared to *Salmonella* Typhimurium strains when grown in 1x TSB at 21°C; (P<0.0001).

Significance: These results indicate that *Salmonella* Heidelberg strains from a recent salmonellosis outbreak may be more likely to form biofilms under stressful (nutrient-limited) conditions, which could contribute to their survival in poultry processing environments.

P2-22 Effects of Boning Time on Bacterial Load of Horse Meat

BRIAN WALKER, Heather Bruce and Lynn McMullen
University of Alberta, Edmonton, AB, Canada

Introduction: The global horse meat market averages 700,000 tonnes per year with the majority being consumed in European and Asian countries. Canadian meat regulations require that the warmest part of a carcass reaches an internal temperature of 7°C prior to boning; however, no scientific data on the impact of early harvest on the microbiology of horse meat is available.

Purpose: To evaluate the impact of boning time on the total aerobic bacteria, lactic acid bacteria, and *Enterobacteriaceae* on horse meat.

Methods: Horse carcass sides (>180kg; n=36) were chilled for 17, 26, or 30 h prior to boning. After chilling, the semimembranosus muscles were harvested from the right side. The entire surface (1036 cm^2) of the muscle was swabbed and enumerated for total aerobic bacteria, lactic acid bacteria and *Enterobacteriaceae*. Muscles were cut into steaks, vacuum packaged and stored for up to 90 days at 0°C. At regular intervals steaks were removed for microbial analysis. Mean log CFU/cm^2 were analyzed with ANOVA.

Results: The time of boning had no effect (P>0.05) on the total aerobic, lactic acid bacteria, or *Enterobacteriaceae* when boned at 17, 26, or 30 h. Total aerobic counts were 1.6±0.4, 1.5±0.4, and 1.7±0.4 log CFU/cm^2, lactic acid bacteria were 0.3±0.5, -0.4±0.7, and 0.0±0.5 log CFU/cm^2, and *Enterobacteriaceae* were -2.2±1.2, -1.7±1.4, and -1.5±1.4 log CFU/cm^2, respectively. Numbers are extremely low due to the large surface area that was swabbed. During storage numbers of organisms decreased initially and then increased during storage. Cell counts only reached a maximum of three log CFU/cm^2 after 90 days of storage.

Significance: Shorter chill times had no adverse effects on the hygienic condition of horse meat allowing horse meat to be harvested at a higher internal carcass temperature without compromising microbial quality and storage life.

P2-23 Risks and Regulatory Approaches Associated with Ready-to-Eat Raw Meat Dishes: A Literature Review and Jurisdiction Scan

Naghmeh Parto[1], **JINHEE KIM**[2] and Ray Copes[2]
[1]Public Health Ontario, Toronto, ON, Canada, [2]Public Health Ontario and University of Toronto, Toronto, ON, Canada

Introduction: Despite evidence on the risks associated with consumption, raw meat dishes such as steak tartare, carpaccio, and kibbe are in demand and widely available in restaurants in many jurisdictions.

Purpose: To support a food safety regulator considering possible approaches to raw meat dishes for retail, we conducted a review of the current evidence on food preparation practices, excluding cooking, which would lower the risk of foodborne illness associated with consumption of ready-to-eat raw meat dishes. We also performed a jurisdiction scan of regulations involving raw meat dishes for sale.

Methods: We performed a search using MEDLINE, Food Science Source and Scopus databases for food preparation methods that reduce the risks associated with ready-to-eat raw meat dishes (excluding poultry and fish), limited to literature published in English from January 1, 1995 to July 10, 2015. The jurisdiction scan was done by a search of the Canadian Legal Information Institute and Google, for regulations and guidance from Canada, United States, United Kingdom and Australia.

Results: Various food preparation methods, from farm to fork, were identified to reduce pathogen load in raw meat. Chemical dehairing of hide, acidified sodium chlorite treatment of trim and high pressure processing are examples. The addition of garlic, lemon juice and yoghurt to raw meat and sear-and-shave procedures have also been shown to reduce microbial load but not eliminate the risk of illness.

General outcome-based or more specific guidance regarding service of raw meat dishes was retrieved for several jurisdictions. Jurisdictions that ban the sale of these food items were also identified.

Significance: Foodborne illness risk can be considerably reduced but not eliminated through practices that reduce bacterial loads on raw meat. Most jurisdictions' approaches involve general outcome-based food safety provisions or specific guidance to mitigate the risks associated with raw meat dishes.

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P2-24 Comparison of Neutralizing Buffered Peptone Water and Dey/Engley Broth in the Recovery of *Salmonella Enterica* from Broiler Carcass Rinsates

Igor Ignatovich, **TERESA PODTBURG**, Oriana Leishman and Scott Steinagel
Ecolab Inc., St. Paul, MN

Introduction: Recently, Food Safety and Inspection Services (FSIS) developed new neutralizing buffered peptone water (nBPW) to replace current buffered peptone water for poultry verification sampling. The use of nBPW is intended to minimize false-negative results in the recovery of pathogenic bacteria.

Purpose: Ecolab has historically utilized a broad spectrum neutralizing Dey/Engley (DE) broth in validation studies. This study was conducted to compare D/E broth to nBPW in neutralizing sanitizer carryover.

Methods: The study evaluated acidified sodium chlorite (ASC) and peroxyacetic acid (PAA) neutralization using D/E and nBPW for whole chicken carcass rinses. PAA and ASC were diluted with the rinsates at 1, 13, and 30 ml representing the zero-, one-, and five-min drip time equivalent volumes, respectively. The solutions were spiked with a 10^5 CFU/ml mixture of five nalidixic acid-resistant *Salmonella enterica* serovars, and incubated at 4°C for 24 h before enumeration analysis using brilliant green sulfa agar supplemented with 100 ppm nalidixic acid. All experiments were performed in triplicates.

Results: *Salmonella* recovery was observed in all samples. PAA performance was similar across the sample types. At zero-min drip time equivalent, the recovery of *Salmonella from* ASC carry-over samples was slightly reduced in nPBW compared to D/E (4.8 log/ml and 5.5 log/ml, respectively), suggesting that antimicrobial activity of ASC may not be completely inhibited by nBPW, and thus can potentially contribute to the false-negative results in the recovery of *Salmonella* during sampling, especially at inadequate drip times.

Significance: These findings show the equivalency or potential advantage of D/E over nBPW. This data highlights the importance of performing neutralization verification in poultry validation studies.

P2-25 Validation of Lactic Acid as an Effective Antimicrobial Intervention for Beef Variety Meats

BYRON CHAVES, Siroj Pokharel, Mark Miller and Mindy Brashears
Texas Tech University, Lubbock, TX

Introduction: Lactic acid is an antimicrobial commonly applied to beef carcasses, primals/subprimals, and ground product to reduce spoilage and pathogenic bacteria. However, little is known about its effectiveness in variety meats.

Purpose: To determine the efficacy of a lactic acid spray on the reduction of microbiological indicators in beef variety meats.

Methods: This study was conducted at a large beef processing plant in the Midwestern United States. Beef heads, hearts, and livers were subjected to a 4.5% lactic acid spay in a designated cabinet. In each of four replicates, 180 sponge samples were collected as follows: 90 before and 90 after antimicrobial intervention, with 30 swabs per meat component and 10 swabs per work shift (morning, midday, evening) being collected for pre- and posttreatment. For each sponge sample, Aerobic Plate Counts (APC), coliform counts (CC), and generic *E. coli*counts (EC) were estimated using 3M Petrifilms®. All Petrifilms® were incubated and read per manufacturer's instructions.

Results: Combined results from four replicates showed that in beef heads APC, CC, and EC were reduced by 2.9, 0.86, and 0.61 log CFU/cm^2, respectively. For livers, the before and after differences for APC, CC, and EC were 1.1, 0.66, and 0.39 log CFU/cm^2, respectively. Lastly, for hearts, average reductions were 1.3, 0.70, and 0.36 log CFU/cm^2, respectively. All differences were deemed significant at a 5% level of significance.

Significance: Foreign markets absorb over 90% of the beef variety meats produced in the United States. Consequently, it is crucial that domestic processors validate and document the microbial safety and quality of their products to maintain the export status.

P2-26 Starter Culture to Inhibit Pore Formation by Heterofermentative Bacteria in Cooked Ham

Jeanne Margerin[1], Zdenek Cech[2] and **VERONIQUE ZULIANI**[1]
[1]CHR HANSEN, Arpajon, France, [2]CHR HANSEN, Milwaukee, WI

Introduction: Pore formation in cooked products, such as ham, is a severe optical drawback for consumers as it is associated with a low quality product. Pore formation can occur due to insufficient vacuum during tumbling or because of contamination with heterofermentative bacteria producing carbon dioxide (CO_2).

Purpose: A challenge test study was conducted by inoculating raw material with a cocktail of CO_2-producing spoilage bacteria in order to demonstrate how a properly selected bioprotective culture can significantly reduce the number and the size of holes in cooked ham.

Methods: Heterofermentative lactic acid bacteria isolated from spoiled cooked ham (*Lactobacillus brevis*) were used to inoculate pork loin at 100 cfu/g. A *Lactobacillus sakei* starter culture was also added into the brine before injection. Tumbling, heating (until reaching 165°F as core temperature), and cooling was then applied to mimic standard industrial ham process. Cooked ham was sliced into two mm thick slices. Slices were scanned and analyzed with ImageJ software to evaluate number and size of pores. An untrained panel was also used to evaluate the ham porosity.

Results: Starter culture *L. sakei* significantly reduced the number and the size of pores caused by microbial contamination of heterofermentative bacteria. Results obtained with ImageJ and the panel are linearly correlated.

Significance: These results suggested that the use of starter culture, as a processing aid, during the manufacturing of cooked meat injected products is an effective hurdle to significantly reduce holes formation due to microbial contamination and, thus, to improve the quality and appearance of the final product.

P2-27 A Multiple Hurdle Carcass Washing Protocol for Inactivating Shiga Toxin-producing *Escherichia coli* on Beef

PRASHANT SINGH and Yen-Con Hung
University of Georgia, Griffin, GA

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 and nonO157 STEC serogroups are important foodborne pathogen affecting beef industry. Cattle hide and gastrointestinal tract are considered the primary source of these pathogens. Beef gets contaminated at the hide removal and evisceration step of the slaughtering process. Effective intervention method is required for lessening the number of STEC related outbreaks in beef.

Purpose: The objective of this study was to develop a multiple hurdle carcass intervention protocol for reducing STEC on beef.

Methods: A 25 cm^2 area on fat and lean side of subprimal beef cuts was marked and were inoculated with (seven or five log CFU/cm^2) five-strain cocktail of nalidixic acid resistant STEC serogroups (O157, O26, O45, O103, and O111). The efficacy of acidic electrolyzed oxidizing water (AEO),

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near-neutral electrolyzed oxidizing water, bleach, lactic acid (4% LA), levulinic acid with sodium dodecyl sulfate (LVASDS 2%:0.02%), Birkoside MP-2 (250 ppm) and acetic acid (160 ppm) was evaluated using a pilot size carcass spray-washing cabinet. Samples after treatment were trimmed and enumerated. Sanitizers showing better reduction were combined to form multiple hurdle intervention protocol.

Results: Individual treatment with AEO, LA, and LVASDS showed 1.7, 3.9, and 1.8 log CFU reductions, respectively. Application of multiple hurdle intervention treatment on beef samples inoculated with seven log CFU/cm^2 resulted in 5.4 and 3.5 log CFU *E. coli* O157/cm^2 reductions on fat and lean side of subprimal beef, respectively. Whereas, samples inoculated with a low concentration (five log CFU/cm^2) resulted in 3.5 log CFU/cm^2 reductions on both meat surfaces. NonO157 STEC serogroups *E. coli* O26, O45, and O103 showed similar reduction trend. However, *E. coli* O111 was significantly (P<0.05) more resistant and showed reduction in range of 1.9 to 3.7 log CFU/cm^2.

Significance: The protocol developed in this study can be used for reducing risk of STEC on beef carcass and trims.

P2-28 Heat Resistance of *Escherichia coli* and *Salmonella enterica* in Ground Beef

BRAD WEBSTER, Michael Gänzle and Lynn McMullen
University of Alberta, Edmonton, AB, Canada

❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* AW1.7 and *Salmonella enterica* serovar Senftenberg ATCC 43845 have comparable heat resistance that is mediated by the locus of heat resistance.

Purpose: The aim of this study was to determine the heat resistance, decimal reduction times (*D*-values), and thermal resistance constants (*z*-values) for *Escherichia coli* AW1.7 and *Salmonella enterica* ATCC 43845 in ground beef.

Methods: Ground beef was inoculated (10^7/g) with *E. coli* AW1.7 or *Salmonella enterica* ATCC 43845, formed into patties (200 g), and cooked to an internal temperature of 71°C. After heating, samples were immediately cooled on ice and plated onto LB agar for enumeration of cells. Inoculated ground beef (10 g) was vacuum packed, immersed in a heated, circulating water bath, and held at 55, 57.5, 60, 62.5, 65, 67.5, and 70°C for different time intervals prior to plating as above. All experiments were done in triplicate. A linear regression model was used to calculate the *D*- and *z*-values.

Results: In ground beef patties cooked to 71°C, *E. coli* AW1.7 and *Salmonella enterica* ATCC 43845 were reduced by 2.9 and 4.0 log CFU/g, respectively. Average *D*-values for *E. coli* AW1.7 and *Salmonella* ATCC 43845 at a temperature range of 55 to 70°C were 33.70 to 0.10 min and 21.14 to 0.14 min, respectively. Calculated *z*-values for *E. coli* AW1.7 and *Salmonella* ATCC 43845 were 5.8 and 6.4, respectively.

Significance: The results of this study can be used by producers to help to assess their process and predict the survival of heat resistant organisms in ground beef products.

P2-29 Evaluating the Potential Nonthermal Microwave Effects of Microwave Irradiation Treatments for Shiga Toxin-producing *Escherichia coli* Decontamination of Fresh and Frozen Beef Intended for Intact and Nonintact Beef Products

DARVIN CUELLAR[1], Don Stull[2], J. Chance Brooks[1], Marcos X. Sanchez-Plata[1], Mindy Brashears[1] and Alejandro Echeverry[1]
[1]Texas Tech University, Lubbock, TX, [2]MicroZap, Lubbock, TX

❖ ***Developing Scientist Competitor***

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) have the ability to produce life-threatening complications. STEC are a preeminent concern for the beef industry, because only small numbers can cause life-threatening damage to organ systems and illness in children, elderly, and other susceptible populations.

Purpose: The overall goal of this project was to evaluate the effectiveness of microwaves as a postharvest intervention to reduce STEC (Serotypes O157:H7, O26, O103, O111, O121, O45, and O145) on vacuum packaged beef stored under refrigerated or frozen conditions.

Methods: Beef strip loins were sectioned into three equal parts, which were randomly assigned to Microwave treatment (MW, treatment using novel microwave with six magnetrons, applying 472.6 kWs of energy per run), control (CTR, inoculated, vacuum packaged, but not treated), or INOC (section used to determine STEC attachment). For each individual microorganism, sections were stored at refrigeration (0 to 4°C) or frozen (-17°C or below). Microbial analyses were made at day 1, 5, and 10 (cold storage) or at 7, 14, and 21 days (frozen storage). Swabs were obtained from each of the beef sections, diluted with 10 ml BPW, and plated onto MacConkey Agar for enumeration. The experiment was replicated three times.

Results: The analysis of variance for each serotype shows statistical significance for storage type with a *P*-value of <0.001 for all serotypes. The samples stored under refrigeration presented statistically higher STEC concentration in CFU/100 cm^2 compared with the ones in frozen storage. Moreover, the samples with serotype O121, O111, O103, O45, and O26, stored under refrigeration, showed a statistical significance for treatment with a *P*-value of 0.01. Only samples with serotype O45 shown statistical significance for treatment, in frozen storage.

Significance: The results showed that under the experimental parameters the microwave treatment was not effective in reducing the STEC on fresh, refrigerated vacuum beef. However, under frozen conditions, STEC concentrations of beef strip loins could potentially be reduced up to 99.9%.

P2-30 Pathogen Reductions in Fermented Dry Sausages Using a Low-temperature Heat Treatment

SAMANTHA MCKINNEY, Catherine Cutter, Nancy Ostiguy and Jonathan Campbell
Penn State University, University Park, PA

❖ ***Developing Scientist Competitor***

Introduction: Meat processors utilize time-temperature combinations found in USDA-FSIS Appendix A as validated support to produce safe, ready-to-eat products. Any deviations from these time and temperature combinations require validation to support the safety of the process by reducing or eliminating pathogenic bacteria.

Purpose: The purpose of this study is to validate the safety of a process that deviates from Appendix A by using a lower temperature at a longer time to produce dried salami. This experiment investigated the impact of processing parameters and casing diameter on the survival of foodborne pathogens in a fermented and dried Genoa salami product.

Methods: Ground pork, starter culture, and non-meat ingredients were mixed, experimentally inoculated with three strains each of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* and stuffed into 38-, 64- and 120-mm fibrous casings (*n*=72). The salamis were then fermented for 7 h at 45°C, heated for 10.5 h at 52°C, dried for 23 d, and vacuum packaged up to 28 d.

Results: A five-log reduction of all three pathogens was achieved for all three diameters by the end of the drying period (23 days). By day 17, a five-log reduction in *E. coli* O157:H7 was achieved for all diameter salamis. A five-log reduction in *Salmonella* spp. was achieved in both the 64- and 120-

mm diameter salamis following the heat treatment, while a five-log reduction occurred in the 38-mm diameter salami after 48 h of drying. A five-log reduction in *L. monocytogenes* was achieved by day 10 of the process for all diameter salamis.

Significance: This study validated the safety of a Genoa salami produced by using alternate thermal processing methods that differ from the required regulatory standards.

P2-31 The Effect of Dipping in Organic Acids for Short or Extended Times on Reduction of *Escherichia coli* Surrogates on Pieced Beef Clods

ASHLEY MCCOY[1] and Dennis Burson[2]

[1]University of Nebraska-Lincoln, Lincoln, NE, [2]University of Nebraska, Lincoln, NE

❖ ***Developing Scientist Competitor***

Introduction: Small business meat processors need simple methods to reduce *Escherichia coli* and shiga toxin-producing *E.coli* (STEC) in ground beef. Organic acid dips may provide controls for STEC.

Purpose: The objective was to evaluate the effect of short or extended dip time and antimicrobial on the reduction of *E. coli* (study 1) and to evaluate the effect of short or extended dip time on lean or fat surfaces on the reduction of *E. coli* (study 2).

Methods: A five-strain cocktail of Rifampicin resistant *E. coli (E.coli*^{RIF}) was inoculated on beef shoulder clod pieces and allowed to attach for 20 minutes. Lean inner surfaces (study 1 and 2) and medial fat surfaces (study 2) were dipped in 4.5% lactic acid (LA, study 1 and 2), or 380 ppm peroxyacetic acid (PAA, study 1) for 15 s or 3 min at 22.2°C. Sample cores (25.3 cm^2) were taken before and after dipping and a 25 g sample after grinding (study 1). Samples were stomached in buffered peptone water containing Rifampicin and dilutions plated on ACP petrifilms (3M; study 1 and 2).

Results: *E.coli*^{RIF} counts pretreatment were not different among treatments. Posttreatment, *E.coli*^{RIF} counts for PAA and LA dipped for three minutes were lower (P<0.05) than the inoculated control. Reduction of *E.coli*^{RIF} counts using PAA at 3 min (0.721 log CFU/cm^2) were also different than inoculated control (0.015 log CFU/cm^2; P<0.05). After grinding *E.coli*^{RIF} counts for LA 15 s, PAA 15 s, and PAA 3 min were all different than inoculated control. No differences in *E.coli*^{RIF} counts for lean or fat surfaces (study 2). However, reductions at three min were greater than reductions at 15 s.

Significance: Reduction levels were greater at longer time periods of dipping. However, if dipped for greater time intervals, product quality could be negatively affected.

P2-32 Application of Bacteriophages to Reduce Shiga Toxin-producing *Escherichia coli* on Beef Cattle Hide Surfaces

TAMRA TOLEN[1], Yicheng Xie[1], Thomas Hairgrove[2], Jason Gill[3] and Thomas Taylor[1]

[1]Texas A&M University, College Station, TX, [2]Texas A&M AgriLife Research, College Station, TX, [3]Texas A&M University, Bryan, TX

❖ ***Developing Scientist Competitor***

Introduction: The cross-contamination of beef products has been attributed to members of the Shiga toxin-producing *E. coli* (STEC), including the O157 and nonO157 STEC. Biopreservation technologies such as lytic bacteriophages can aid the protection of beef safety by reducing STEC on cattle prior to harvest.

Purpose: The objective of this study was to determine if application of lytic bacteriophages reduced the loads of O157 and nonO157 STEC on inoculated cattle hides under conditions simulating live animal intervention application.

Methods: Hide pieces (65.6±6.5 cm^2) were inoculated with a gelatin-based slurry containing a cocktail (9.7±0.1 log CFU/ml) of *E. coli* serotypes O157:H7, O26:H11, O45:H2, O111, O121:H19, and O145. After a 30 min attachment, excess slurry was removed by scraping gelatin off the surface, and hides were treated with a water or phage intervention by a hand-held trigger spray bottle applying treatment. A commercial phage intervention containing 10^8 PFU/ml phages was diluted to 10^7 PFU/ml and compared to an untreated controls and sterile distilled water. The intervention was allowed to dwell for 60 min at 37°C, after which surviving STEC were enumerated from hide pieces on tryptic soy agar supplemented with rifampicin and cycloheximide (100 mg/ml each). Plating of STEC survivors was completed following centrifugation and suspension of STEC in fresh diluent to separate nonadsorbed lytic phages from noninfected STEC.

Results: The mean number of STEC attaching to inoculated hides was 6.8±0.1 log CFU/cm^2 prior to intervention application across two replications (n=6); mean phage titer was 8.4±0.8 log PFU/ml. Mean counts of STEC posttreatment were 6.8±0.1 log for H_2O and phage treatments, respectively (P>0.05); no effective reduction in STEC by either treatment was observed compared with the control.

Significance: Although no differences were detected between positive control hide samples compared to treated hide samples, phages may enhance beef safety, though further optimization of phage interventions is still required.

P2-33 Neutralization of Commercial Broiler Carcass Antimicrobials by Phosphate Buffered Saline, Buffered Peptone Water, and Neutralizing Buffered Peptone Water

JENNIFER VUIA-RISER, Christine Alvarado and Matt Taylor

Texas A&M University, College Station, TX

Introduction: Poultry products have been repeatedly identified as transmission vehicles for *Salmonella enterica*. Poultry processors have incorporated antimicrobial interventions to assist in reducing *Salmonella* on fresh carcasses and parts. Nonetheless, the use of antimicrobial interventions may interfere with detection of *Salmonella* during testing due to carryover of antimicrobials into rinse solutions.

Purpose: The purpose of this study was to compare phosphate buffered saline (PBS; control), buffered peptone water (BPW) and neutralizing buffered peptone water (nBPW) rinse fluids to neutralize antimicrobial carryover on whole broiler carcasses and parts sets during commercial harvest and fabrication.

Methods: Peroxyacetic acid dip (PAA), applied to parts, and cetylpyridinium chloride (CPC) spray and chill-dip, applied to whole carcasses, were applied at 0.06%±0.03% (v/v) and 0.50%±0.06% (v/v), respectively. At random intervals, carcasses (n=20) and parts sets (n=14) were collected for testing; parts sets consisted of four pound samples of wings (three), thighs (three), breasts (four) and drumsticks (four). The United States Department of Agriculture method for the detection of *Salmonella* was utilized for pathogen enrichment and detection.

Results: Mean presumptive-positive *Salmonella* recovery rates for PBS (control), BPW, and nBPW for chicken carcasses were 0%, 0%, and 29.2%, respectively, while those for PBS, BPW, and nBPW for chicken parts were 11.9%, 10.7%, and 14.3%, respectively. Recovery rates for presumptive-positive *Salmonella* on whole carcass nBPW was higher (P<0.001) than PBS and BPW. Statistical analysis indicated no differences in presumptive-positive *Salmonella* recoveries for chicken parts sets as a function of rinse fluid (P=0.47).

Significance: Results of testing indicate the null hypothesis must be rejected for carcasses but could not be rejected for parts, in that nBPW was not apparently more effective at aiding *Salmonella* recovery versus BPW. These do not provide strong evidence nBPW is more effective for antimicrobial neutralization during poultry carcass or parts rinsing versus BPW.

P2-34 Validation of a Chicken Surface Methodology to Assess the Performance of Antimicrobial Interventions on Chicken Parts

ALEJANDRA RAMIREZ-HERNANDEZ, Mindy Brashears and Marcos X. Sanchez-Plata
Texas Tech University, Lubbock, TX

Introduction: The poultry industry in the United States. has to demonstrate control of *Salmonella* and *Campylobacter* prevalence in chicken parts and comminuted products as part of USDA performance standards. A series of antimicrobial interventions have been implemented at different stages of processing with mixed results. Unfortunately, the application in commercial settings does not allow processors to understand the effects of the intervention at the surface level.

Purpose: A validated methodology to simulate processing conditions and evaluate interventions under the conditions of processing stages is needed. A chicken surface coupon methodology was evaluated for this project using chicken skin and chicken meat surfaces to compare the efficacy of antimicrobial solutions on inoculated chicken pieces under simulated commercial conditions.

Methods: Fresh chicken skin and meat coupons (4 cm^2) were inoculated with 20 µl of a five strains of *Salmonella* spp. cocktail and placed side up to allow attachment (20 min). Samples were subjected to antimicrobial applications, including lactic acid (LA; 5% v/v), cetylpyridinium chloride (CPC; 0.8% w/v) and peracetic acid (PPA; 400 ppm). The solution was sprayed on the top side of the chicken skin/meat for complete coverage. After treatment, each coupon was placed in a tube containing nine ml of nBPW and homogenized. Serial dilutions were made on BPW and dilutions were spread plated on XLD agar with TSA overlay. Plates were incubated for 24 h at 37°C. Plates were enumerated and bacterial populations were converted to log CFU/cm^2. Additional chicken skin/meat pieces were subjected to scanning electron microscopy to identify bacterial attachment and viability.

Results: All interventions had an effect by reducing *Salmonella* attachment on chicken coupons. There was a statistical significant bacteria reduction ($P<0.05$) on the chicken meat coupons (0.85 log CFU/cm^2) treated with LA, but nonstatistical significant on chicken skin.

Significance: There is a need to screen the efficacy of antimicrobial interventions before selecting effective treatments for implementation. This methodology could facilitate this process for commercial operations.

P2-35 Validation of Fermentation, Drying, and Storage Parameters for Control of Shiga Toxin-producing *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes* in Fuet, a Traditional Spanish Sausage

ANNA PORTO-FETT[1], Esteve Sargatal[2], Laura Shane[1], Lianna McGeary[1], Bradley Shoyer[1], Laura Stahler[1], Manuela Osoria[1] and John Luchansky[1]
[1]U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, [2]Espuña LLC, Gloversville, NY

Introduction: *Salmonella* spp. (Sal), Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* (Lm) may survive during manufacture and storage of many types of specialty-ethnic ready-to-eat meats. Thus, research is warranted to further reduce the risk of foodborne illnesses associated with the consumption of these specialty meat products.

Purpose: Evaluate the effectiveness of processing parameters on the viability of cells of Sal, Lm, and STEC in fuet.

Methods: All-pork (30% fat) coarse ground meat was mixed with salt (2.5%), starter culture, and spices, and then separately inoculated with a multistrain cocktail (ca. 6.8 log CFU/g) of STEC, Sal, or Lm. The batter was stuffed into a 55-mm natural casing and subsequently fermented at 23±2°C and ca. 95±4% relative humidity (RH) to a final target pH of pH≤5.3. Sausage was then dried at 12±2°C and ca. 75 to 85% RH to a final target water activity of a_w 0.89 or a_w0.86. Next, product was vacuum-packaged and stored for up to 30 days at 20±1°C (n=3 trials, n=3 samples per trial).

Results: Overall, pathogen numbers remained relatively unchanged after fermentation, but reductions of ca. 1.1 to 2.8 and ca. 2.7 to 4.1 log CFU/g were achieved at the end of drying and after storage, respectively. Total reductions of ca. ≥5.3 log CFU/g in *Salmonella*, STEC, and Lm numbers were achieved after fermentation, drying, and storage in fuet dried to an a_w of 0.86. For sausage dried to an a_w of 0.89, total reductions of ca. 5.3 log CFU/g in STEC or *Salmonella* numbers were achieved after fermentation, drying, and storage, whereas a total reduction of ca. 4.1 log CFU/g in Lm numbers was achieved.

Significance: The processing parameters tested herein validated that it was possible to achieve the required 2.0 or 5.0 log reductions in levels of Sal, Lm, and/or STEC during manufacture of fuet.

P2-36 Biofilm Forming Capabilities of Shiga-toxigenic *Escherichia coli* Recovered from Cow/Calf Operations in Oklahoma and Louisiana

TONY KOUNTOUPIS[1], Pushpinder Kaur Litt[1], Radhika Kakani[2] and Divya Jaroni[1]
[1]Oklahoma State University, Stillwater, OK, [2]Oklahoma State Univsersity, Stillwater, OK

✦ ***Undergraduate Student Award Competitor***

Introduction: Shiga-toxigenic *Escherichia coli* (STEC) is one of the most common groups of foodborne pathogens and has been associated with foodborne outbreaks throughout the United States. The ability to form biofilms on both food and food processing equipment contributes to STEC persistence, as well as the number of widespread outbreaks. This shows the importance of characterizing STEC biofilms and understanding how these bacteria travel through the food supply chain from pre-harvest to consumer goods.

Purpose: Determine biofilm forming capabilities of STEC isolates recovered from cow calf operations.

Methods: Seven STEC serogroups (O157, O26, O45, O103, O111, O121, O145) were tested for their biofilm capability. Of the several wild-type (WT) STEC, isolated from cattle operations in Oklahoma, 14 (O157-LF4, O157-KF10, 0157-JEQ1, O26-QF6, O26-BF8, O26-AF5, O45-HF9, O45-AF1, O45-EF2, O103-SF2, O103-GF8, O103-AF10, O121-GF6, O145-BF9) were tested for their biofilm forming capacity along with seven lab strains (O157-43895, O121, O111, O103, O26, O145, O45). Microtiter plates (96-well) were inoculated with ~ seven log CFU/ml of the aforementioned STEC, and incubated for 24 h to allow for biofilm formation. After incubation, unattached cells were removed; wells washed with phosphate buffered saline; and stained using crystal violet. The crystal violet stain was released in ethanol:acetone solution and absorbance was recorded at 595 nm.

Results: Based on the absorbance, the biofilm-forming capability of tested STEC strains was categorized as follows: Very Strong ($A_{595}>0.9$), Strong ($0.9<A_{595}> 0.6$), Medium ($0.6<A_{595}>0.3$), Weak ($0.3<A_{595}>0.1$) and nonbiofilm formers ($A_{595}<0.1$). All 21 STEC strains were found to be capable of biofilm formation. All STEC (lab and WT) strains produced very strong biofilms, except O111 (lab) and O103-SF2 (WT), which were only capable of strong (0.916 and 0.887 respectively) biofilm formation.

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Significance: Identifying and measuring the biofilm forming capability of STEC strains can lead to the development of better intervention strategies for the food industry.

P2-37 The Effect of Preharvest Feeding Strategies on the Prevalence of *Salmonella enterica* in the Feces and Trimmings of Feedlot Cattle

KATLYN HOLZER[1], Carla Weissend[1], Jennifer Martin[2], Kate Huebner[1], Ifigenia Geornaras[2], Paul Morley[1] and Keith Belk[2]
[1]*Colorado State University, Ft Collins, CO,* [2]*Colorado State University, Fort Collins, CO*

❖ ***Developing Scientist Competitor***

Introduction: There is pressure in agriculture to reduce the use of antimicrobials in livestock production. Tylosin, a macrolide antimicrobial, is commonly used for prevention of liver abscesses in cattle. As macrolides are important to human medicine, efforts are being made to reduce their use in agriculture. Natural compounds have shown promise as alternatives for macrolides in beef finishing diets and their impact on pathogen prevalence in the beef supply chain is worthy of investigation.

Purpose: This study evaluated the influence of including naturally-derived feed additives in beef cattle finishing diets on the presence of *Salmonella enterica* in feces and beef trimmings.

Methods: Commercial cattle in northern Texas (*n*=5,481 head) were randomly assigned to one of four treatment groups with 10 pen blocks per treatment. The finishing ration treatment groups included (i) with tylosin; (ii) without tylosin; (iii) without tylosin, but with an essential oil (CRINA-L; source of limonene); and (iv) without tylosin but with a yeast fermentation byproduct. Pen-floor fecal samples were collected from each pen. Finished cattle were harvested at a commercial processing facility and trimmings were collected from each pen at fabrication. *Salmonella enterica* was isolated from composite fecal and trim samples using standardized methodologies.

Results: Thirty-eight of the 40 fecal samples (95%) and, as expected for this region, 11 of 36 composited trimmings samples (31%) were positive for *Salmonella.* Fecal *Salmonella* prevalence did not differ among treatment groups (*P*>0.05). No correlation existed between fecal sample positives for *Salmonella* and trimmings samples that also were positive for *Salmonella*.

Significance: As alternatives for antibiotics in finishing cattle diets are explored, their influence on pathogen presence in the beef supply chain is important. These data suggest that inclusion of yeast fermentation extracts or essential oils in finishing rations has little influence on the presence of *Salmonella* in feces or trimmings.

P2-38 *Salmonella* and *Campylobacter* Prevalence in Broiler Ceca and on Ready-to-Cook Carcasses Processed at a Pilot Mobile Poultry Processing Unit

KA WANG LI, Lacey Lemonakis, Brian Glover and Cangliang Shen
West Virginia University, Morgantown, WV

❖ ***Developing Scientist Competitor***

Introduction: S*almonella* and *Campylobacter* are concerns for mobile poultry processing unit (MPPU) produced carcasses due to the exemption of USDA-FSIS poultry products inspection act.

Purpose: This study aims to evaluate the *Salmonella* and *Campylobacter* prevalence in broiler ceca and MPPU processed ready-to-cook carcasses.

Methods: Straight-run Hubbard x Cobb broilers were reared for 38-day on clean shavings or built-up litter. A total of 139 carcasses (68 from clean shavings and 71 from built-up litter) were processed at a MPPU facility. For *Salmonella*, ceca and carcasses were preenriched (buffered peptone water), secondly-enriched (Rappaport-Vassiliadis medium) and streak-plated onto XLT-4 and HardyCHROM™-agar, and further confirmed by API 20E kit. For *Campylobacter*, ceca and carcasses were enriched in Bolton broth and on modified-campy-cefex-agar under microaerophilic conditions (5.0% O_2, 10% CO_2, and 85% N_2) at 42°C for 48 to 72h, and further confirmed using *Campylobacter* Latex Test Kit and Gram staining. A qPCR-test (CadF-gene) was conducted to identify the prevalence of *Campylobacter jejuni* and *Campylobacter coli* in ceca and on carcasses. Data (three replicates/~23 to 26 samples/replicate) were analyzed using Chi-Square of SAS.

Results: *Salmonella* was not detected in any of the cecal samples, while *Salmonella* was present in 2.8% of the carcasses from the built-up litter. The prevalence of *Campylobacter* decreased (*P*<0.05) in ceca (64.6% vs. 84.6%) and carcasses (50% vs. 56.3%) obtained from broilers reared on clean shavings compared to those reared on built-up litter. The prevalence of *Campylobacter jejuni* was lower (*P*<0.05) on clean shaving treated birds ceca (20.0% vs. 27.3%) and carcasses (22.2% vs. 42.2%) than built-up litter samples. However, *Campylobacter coli* was present higher (*P*<0.05) in clean shavings birds ceca (31.8% vs. 27.3%) and carcasses (65.1% vs. 37.8%) than built-up litter treated samples.

Significance: Raising broilers on clean shavings as opposed to built-up litter may decrease the presence of foodborne pathogens. But postharvest antimicrobial treatment is still necessary to control *Campylobacter* on MPPU produced broiler carcasses.

P2-39 Isolation and Characterization of Extended-spectrum Beta-lactamase-producing *Escherichia coli* from Beef Cattle Farms

SHINYOUNG LEE, Lin Teng, JaeHyun Lim, JungHoon Park and KwangCheol Casey Jeong
University of Florida, Gainesville, FL

Introduction: The global dissemination of extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) threatens public health. Although it is controversial, food-producing animals contribute to the burden of ESBLs. However, limited information is available regarding the prevalence of ESBLs on beef cow/calf operations.

Purpose: The objective of this study was to analyze the prevalence of ESBL-producing *Enterobacteriaceae* (ESBL-*E*) and ESBL-*E. coli* on cow/calf operations located in Florida to understand the spread of ESBLs among food animals and to characterize the ESBLs isolated from animals.

Methods: One thousand ninety-six samples were collected from 17 farms including feces of calves and cows, soil, water, and forage. ESBL-*E* and ESBL-*E. coli* were isolated by plating on MacConkey agar containing cefotaxime (four μg/ml) and ChromAgar *E. coli* media. The prevalence was calculated by dividing the number of positive samples by the total number of samples and the confidence interval (CI) was estimated with a 95% confidence level. The ESBL genes were identified using PCR. Minimum inhibitory concentration (MIC) to cefotaxime and antibiotic susceptibility against 13 different antibiotics were determined to characterize ESBL-*E.coli*.

Results: All the farms had ESBL-*E*, the prevalence ranged between 13.15% and 63.63%. The average concentration of ESBL-*E* was 1.68 log CFU/g of feces (95% CI: 1.6 to 1.77). Sixty-five percent (11 of 17) of beef farms had ESBL-*E. coli* in the majority of the samples (feces, forage, and soil), except

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water sample. The average prevalence and concentration were 7.42% and 1.56 log CFU/g of feces (95% CI: 1.37 to 1.74), respectively. The CTX-M gene was the most predominant ESBL gene type. Furthermore, all isolates showed a MIC of cefotaxime ≥16 µg/ml and multidrug resistance, suggesting that it could be a potential life-threatening factor.

Significance: Our results provide critical knowledge to better understand the prevalence and characteristics of ESBLs on cattle operations in Florida where antibiotics were not extensively used for prophylactic purpose.

P2-40 Near-neutral Electrolyzed Oxidizing Water Applied as Postharvest Intervention to Control *Escherichia coli* O157:H7, *Salmonella* Enteritidis and *Yersinia enterocolitica* in Fresh Pork

DONG HAN[1], Yen-Con Hung[2] and Luxin Wang[1]

[1]Auburn University, Auburn, AL, [2]University of Georgia, Griffin, GA

Introduction: A total of 639,642 pounds of pork were recalled in 2015 due to a *Salmonella* outbreak. Pathogenic *Escherichia coli*, *Salmonella* spp., and *Yersinia enterocolitica* are considered to be major foodborne pathogens in pork products. Near-neutral electrolyzed oxidizing (NEO) water has been proven to be an efficient post-harvest intervention method for several food commodities.

Purpose: The objective of this study is to evaluate the bactericidal efficacy of NEO water on *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Yersinia enterocolitica* in pork.

Methods: One milliliter of each pathogen was cultured overnight (eight log CFU), washed and treated by adding 10 ml of NEO water (pH: 7.64, oxidation-reduction potential: 818 mV, free chlorine: 74 ppm). Bactericidal efficiency was evaluated by plating bacterial cultures on selective media after 15, 30, and 60 seconds of treatment. Freshly-harvested pork skin and chops (5 by 5 cm) were inoculated with target pathogens at final inoculation levels of four to five log CFU/cm^2. Samples were treated with NEO water for either two or 10 minutes and plated for bacteria enumeration.

Results: In the pure culture study, bacterial populations dropped below the limit of detection (two log CFU/ml) after 30 s of treatment. No live cells were recovered with enrichment after the 60 s treatment. Significant bacterial population decreases (P<0.05) were observed from all treated inoculated pork skins, the reductions were 2.59 log CFU/cm^2 for *E. coli* O157:H7, 2.36 log CFU/cm^2 for *Salmonella* and 1.81 log CFU/cm^2 for *Yersinia*. The reductions on pork chops were 0.29 log CFU/cm^2 (P<0.05) for *E. coli* O157:H7, 0.25 log CFU/cm^2 for *Salmonella* and 0.14 log CFU/cm^2 (P<0.05) for *Yersinia*.

Significance: Our results suggest that NEO water has the great potential to be used as a spray treatment for carcasses and pork products.

P2-41 Withdrawn

P2-42 Effect of *Salmonella* Vaccine Strains on Broiler Chicken Cecal Microbiota

SI HONG PARK[1], Sun Ae Kim[1], Peter Rubinelli[1], Christopher Baker[2] and Steven Ricke[1]

[1]University of Arkansas, Fayetteville, AR, [2]University of Florida, Gainesville, FL

Introduction: Vaccination is one of the more effective means for controlling *Salmonella* in poultry. The chicken ceca are fermentation chambers involved in polysaccharide digestion, water adsorption and urea recycling as well as possessing a wide range of indigenous microbial organisms. Although microbiota in the poultry ceca may play an important role in host health, a significant lack of knowledge exists regarding the effect of vaccine strains on normal microbiota in chicken ceca.

Purpose: Evaluation of microbial composition changes in chicken cecal contents after vaccine administration to assess potential impacts of the *Salmonella* Typhimurium vaccine strains.

Methods: The cecal microbial communities of unvaccinated (group A) and vaccinated chickens (group 2, vaccinated with P_{BAD}-*mviN*; group 3, vaccinated with wild type; group 4, vaccinated with DD*metRmetD*) were compared through denaturing gradient gel electrophoresis (DGGE) and microbiome sequencing analysis.

Results: Chickens from different treatment groups exhibited DGGE patterns that were indistinguishable from each other. However, microbiome sequencing analysis of individual chicken ceca revealed considerable changes in microbial composition by different vaccine strain treatments and detectable patterns of distinctive clustering among groups. The P_{BAD}-*mviN Salmonella* Typhimurium yielding the highest vaccine efficacy led to the most distinctive changes in the cecal microbiota.

Significance: The present study provides comprehensive information about the impact of vaccine strains which can effectively control *Salmonella* Typhimurium in poultry on the microbiota of host and it may contribute to better understanding of vaccine strain effects.

P2-43 Effect of Sodium Chloride on the Heat Resistance of Enterohemorrhagic *Escherichia coli* in Ground Beef

ZIYI HU, Alina Rohde, Jiayue Chen, Lynn McMullen and Michael Gänzle

University of Alberta, Edmonton, AB, Canada

❖ ***Developing Scientist Competitor***

Introduction: Cattle are a reservoir for enterohemorrhagic *Escherichia coli* (EHEC), and ground beef is a significant vehicle for human infection with EHEC. Heat resistance of *E.coli* including EHEC is variable and survival of *E.coli* after heating in broth is impacted by sodium chloride (NaCl) and other additives.

Purpose: This study aimed to evaluate the effect of NaCl and other additives on the heat resistance of *E.coli* in ground beef.

Methods: The heat resistant *E. coli* AW1.7ΔpHR1-pLHR, the isogenic heat sensitive *E. coli* AW1.7ΔpHR1-pRK767, or a five-strain cocktail of EHEC were inoculated (10^7 CFU/g) into ground beef (15% fat) with 0, 1, or 3% NaCl. Patties were grilled immediately, or stored in sterile bags for two days at 4°C prior to grilling to a core temperature of 71°C. Cores from patties were cooled on ice prior to plating on LB agar. Experiments were performed in biological triplicates and statistical significance was evaluated using one-way ANOVA.

Results: Grilling of patties reduced counts of heat resistant strain *E. coli* AW1.7ΔpHR1-pLHR by 3.43 ± 0.32 log cfu/g; the heat sensitive *E. coli* AW1.7ΔpHR1-pRK767 and the EHEC cocktail were reduced by more than six log cfu/g. Addition of 3% NaCl significantly (P<0.05) increased survival of *E. coli* AW1.7ΔpHR1-pRK767 and the EHEC cocktail while counts of the heat resistant strains were not changed. A protective effect of NaCl was not observed with *E.coli* AW1.7ΔpHR1-pRK767 if cells of *E. coli* were cooled to 4°C prior to mixing with cold meat and NaCl, indicating that the response of *E. coli* to osmotic shock contributes to this effect.

Significance: Heating of patties to 71°C may reduce counts of EHEC by less than five log cfu/g if cells are adapted to NaCl. The effect of refrigerated or frozen storage on heat resistance of EHEC in ground beef remains subject to future investigations.

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P2-44 Characterization of Attachment Differences of Shiga Toxin-producing *Escherichia coli* to Prechill and Postchill Beef Tissues

BENNETT UHL[1], Daniel Unruh[1], Randall Phebus[2] and Sara Gragg[1]
[1]*Kansas State University, Olathe, KS,* [2]*Kansas State University, Manhattan, KS*

✦ ***Undergraduate Student Award Competitor***

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) has been implicated in beef-related foodborne illness outbreaks. Environmental factors influence bacterial attachment on beef and understanding bacterial attachment may inform future interventions at the abattoir.

Purpose: This study measured STEC attachment under simulated meat processing conditions on adipose and lean beef tissue.

Methods: Beef brisket was purchased from a local grocer, and 50 cm^2 adipose and lean tissue samples were obtained and stored overnight (18 h; 4°C). The following day, half of the samples were heated to a surface temperature of 30°C while the remaining samples were maintained at 4°C prior to inoculation with 150 µL STEC cocktail (O26, O45, O103, O111, O121, O145, and O157:H7; ca. 7 log CFU/mL) onto the meat surface. Samples were stored at 4°C 30 min after inoculation and enumerated at times 0, 3, 5, and 20 min and 1, 3, 8, 12, 24 and 48 h by spread plating loosely attached cells (buffer) and firmly attached cells (homogenized sample) on MacConkey Agar. At every sampling point, each meat sample was shaken for 90 s in a stomacher bag with 0.1% peptone water (PW), transferred into a second stomacher bag with fresh PW, and homogenized.

Results: Time*sample type (buffer vs. homogenized sample) was significant ($P \leq 0.001$), as STEC cells steadily became more firmly attached throughout the 48 h storage period. Sample type*meat type was statistically significant ($P = 0.0020$) indicating a difference in loose vs. firmly attached populations on lean and adipose tissues; however, the largest difference observed was 0.22 log CFU/g.

Significance: These data demonstrate that the firmly attached STEC population steadily increases on lean and adipose beef tissues over time. Future research should investigate if an increase in firmly attached STEC cells is correlated to reduced intervention efficacy on post-chill carcasses and subprimal cuts, as commonly observed.

P2-45 Transfer of *Listeria innocua* Biofilm Cells and Regrowth in Duck Meat

Hye Ri Jeon, **MIJIN KWON**, Hye Jin Moon and Ki Sun Yoon
Kyung Hee University, Seoul, South Korea

Introduction: The contamination rate of *Listeria innocua* is higher than that of *Listeria monocytogenes* in poultry carcasses and food production environments. The biofilm formation ability of *L. innocua* is also higher than that of *L. monocytogenes* at various temperatures. Moreover, biofilm formed on food contact surfaces is a potential hazard leading to cross-contamination during food processing.

Purpose: The objective of this study was to investigate the transfer rate of *L. innocua* biofilm cells from food contact surfaces to food and the growth potential of biofilm cells on foods.

Methods: *Listeria innocua* biofilms were formed on four kinds of materials (rubber, polypropylene, glass, and stainless steel), which were put in contact with cooked duck meat (boiled or baked) for five min. Cooked duck meat with transferred biofilm cells was stored at 4°C and 10°C. The growth of biofilm and planktonic cells on cooked duck meat were compared. The viability of biofilm cells formed on food contact surfaces and of transferred cells to duck meat were also confirmed by fluorescence microscope.

Results: The highest transfer rate of *L. innocua* biofilm cells was observed in the polypropylene material and the lowest was in the stainless steel material, regardless of the cooking method of duck meat. The viability of transferred biofilm cells to duck meat was confirmed. Although the lag time of biofilm cells was delayed compared to planktonic cells, the transferred biofilm cells were observed to grow to the maximum population density.

Significance: Biofilm cells from food contact surfaces transferred to food due to cross-contamination and the possibility of growth can be a risk at foodservice industry. Thus, it is important to apply an effective sanitizing step to prevent and reduce the biofilm formation on food contact surfaces during food preparation.

P2-46 Effectiveness of Sanitizer D7™ against *Escherichia coli* O157:H7 and *Salmonella* Biofilms

RONG WANG[1], Norasak Kalchayanand[1], Dayna Brichta-Harhay[1] and You Zhou[2]
[1]*U.S. Department of Agriculture-ARS, Clay Center, NE,* [2]*University of Nebraska-Lincoln, Lincoln, NE*

Introduction: Biofilm formation by *Escherichia coli* O157:H7 and *Salmonella enterica* at meat processing plants poses a serious risk of meat product contamination. Available studies have shown that many common sanitizers were unable to completely eradicate biofilms by these foodborne pathogens due to the three-dimensional biofilm structure and the bacterial polymeric extracellular substances (EPS). D7™ is a sanitizer product consisting of quaternary ammonium compound, hydrogen peroxide, and an optional accelerator, diacetin. This product has been tested on *Listeria* and *Pseudomonas* biofilms and showed promising results. However, the effectiveness of D7™ against *E. coli* O157:H7 and *Salmonella* biofilms under meat processing conditions has not been evaluated.

Purpose: To evaluate the effectiveness of D7™ in inactivating and removing *E. coli* O157:H7 and *Salmonella* biofilms.

Methods: *Escherichia coli* O157:H7 and *Salmonella* biofilms on food contact surfaces were treated with 10, 20, or 100% D7™ for 10 min, 1 h, or 6 h to measure log reduction of biofilm cells. Scanning electron microscope (SEM) was applied to evaluate the effect of D7™ treatment on biofilm removal and bacterial morphology.

Results: Treatment with 10, 20, or 100% D7™ reduced biofilm cells to nondetectable level, even with 10 min exposure time. SEM analysis of water-treated control samples exhibited three-dimensional biofilm structure with strong EPS matrix connecting bacteria with contact surface. Treatment with 20% D7™ for 10 min significantly reduced biofilm mass and weakened EPS connection. Majority of the bacteria exhibited altered morphology with compromised membrane integrity. Treatment with 100% D7™ for 10 min dissolved EPS matrix and no intact biofilm structure was observed, instead, scattered clusters of bacterial aggregates were detected, indicating the loss of cell viability and biofilm removal.

Significance: D7™ is highly effective, even at significantly diluted concentrations, against *E. coli* O157:H7 and *Salmonella* biofilms.

P2-47 Molecular Genotyping and Biofilm Formation of Enterotoxigenic *Clostridium perfringens*

OK KYUNG KOO and Wensi Hu
Gyeongsang National University, Jinju, South Korea

Introduction: Foodborne illness by *Clostridium perfringens* has been dramatically increased in South Korea being the third most prevalent pathogen in the last five years, followed after pathogenic *Escherichia coli* and norovirus. However, only limited studies have been done on molecular epidemiology and survival on food processing facilities of this pathogen.

Purpose: The objective of this study was to investigate the prevalence of *C. perfringens* in South Korea and the biofilm formation ability by toxin type.

Methods: Raw beef (*n*=74), pork (*n*=82) and poultry (chicken, *n*=62; duck, *n*=8) were collected from food service facilities in three different regions of Gyeongsangnam-do in South Korea. *Clostridium perfringens* were isolated by cooked-meat medium incubated for 24 h at 37°C under anaerobic condition and transferred to tryptose sulphite cycloserine with D-cycloserine and egg yolk emulsion. Colonies with lecithinase positive and black color were selected as positive. PCR was performed for toxin typing with specific primers to toxin genes (*cpe, cpa, cpb, cpb2, etx, iap*). Crystal violet assay was performed to evaluate the biofilm formation abilities of the isolates and their correlations with toxin type and sampling region.

Results: Out of 226 samples, 60 samples were positive (24.3% in raw beef, 12.2% in pork, 41.9% in chicken and 75% in duck) and the average concentration in raw meat was 176 CFU/g. All isolates were positive in *cpa* to be toxin type A and enterotoxin gene *cpe* and beta2 gene *cpb2*-positive isolates were confirmed among the isolates. The contamination level and toxin type were closely related to geography of the samples. Also, biofilm formations of the isolates were relatively weak while few isolates showed strong biofilm forming abilities that were more resistant to disinfectants.

Significance: The results showed the most prevalent toxin type of *C. perfriengens* and their correlation between biofilm formation and toxin types to control in the food processing environment.

P2-48 Distribution and Virulence of *Salmonella* spp. Obtained from a Pork Chain Production in Brazil

Frederico Germano Piscitelli Alvarenga Lanna[1], Bruna Torres Furtado Martins[1], Danilo Augusto Lopes Silva[1], Clarisse Vieira Botelho[1], Juliana Libero Grossi[1], Ricardo Seiti Yamatogi[1], Luciano dos Santos Bersot[2] and **LUIS NERO**[3]

[1]Universidade Federal de Viçosa, Viçosa, Brazil, [2]Universidade Federal do Paraná, Palotina, Brazil, [3]Universidade Federal de Viçosa, Vicosa, Brazil

Introduction: *Salmonella* spp. is widespread in the pork production chain, being naturally found in healthy animals that act as reservoirs. Considering this characteristic, the control of *Salmonella* spp. must be conducted in all steps of the pork production. In addition, the characterization of virulence markers in *Salmonella* spp. obtained from pork is relevant to predict the risks to consumers.

Purpose: This study aimed to verify the distribution of *Salmonella* spp. along a pork production chain, including farming and slaughtering, and to characterize virulence markers in the obtained isolates.

Methods: Two pig farms and a slaughterhouse were selected for sampling different production steps of ten pig lots, resulting in 670 samples (finishing barns: 20, swine carcasses: 400, equipment and utensils: 180, end products: 70). Samples were subjected to *Salmonella* spp. detection according ISO 6579, and suspect isolates were subjected to PCR targeting *invA* and *ompC*. *Salmonella* spp. isolates were subjected to PCR to detect 15 virulence related genes (despite *invA*).

Results: In pig farms, nine finishing barns (45.0%) were *Salmonella* spp. positive, while only end products in the slaughterhouse, specifically fresh sausages, presented the pathogen (*n*=6, 8.6%). A total of 42 *Salmonella* spp. isolates were identified by presenting *invA* and/or *ompC* (barns: 17; sausages: 25). Different frequencies of virulence related genes were identified among isolates, being *cdtB* (*n*=13, 31.0%) and *iroN* (*n*=23, 54.8%) the less frequent, while *sitC*, *pagC*, *prgH*, *sopB* and *spaN* were detected in all isolates (*n*=42, 100%); *invA*were identified in 35 (83.3%) isolates. Different associations of positive results for virulence genes were recorded, and a minimum of 10 genes were identified simultaneously per isolate.

Significance: The results indicate the relevance of *Salmonella* spp. in the final steps of pork production and the potential risks of this pathogen to consumers.

P2-49 Tracking of *Salmonella* spp. Contamination Routes in a Pork Production Chain in Brazil

Luciano dos Santos Bersot[1], Valéria Cavicchioli[2], Raquel Burin[2], Cibeli Viana[2], José Paes de Almeida Nogueira Pinto[3], **LUIS NERO**[4] and Maria Teresa Destro[5]

[1]Universidade Federal do Paraná, Palotina, Brazil, [2]Universidade Federal de Viçosa, Viçosa, Brazil, [3]Universidade Estadual Paulista, Botucatu, Brazil, [4]Universidade Federal de Viçosa, Vicosa, Brazil, [5]bioMérieux, Inc., São Paulo, Brazil

Introduction: *Salmonella* spp. is a relevant foodborne pathogen associated to a diversity of cases and outbreaks related to the consumption of pork products. Considering the characteristics of swine production, *Salmonella* spp. can be present in the pork chain since the beginning of production, in piglets farms, until the processing of final products in slaughterhouses.

Purpose: This study aimed to identify the main contamination sources of *Salmonella* spp. in pork production chain in Brazil.

Methods: Six lots of piglets produced in different farms were tracked until their slaughtering, being samples collected from piglets production farms (*n*=662), pigs finishing farms (*n*=636) and slaughterhouse (*n*=270). The samples (feed, water, floor, feces, carcasses, lymph nodes, utensils, equipment) were subjected to *Salmonella* spp. detection according to USDA protocol. Isolates were serotyped and subjected to pulsed-field gel electrophoresis (PFGE) after macrorestriction with *Xba*I.

Results: *Salmonella* spp. was detected in 160 (10.2%) samples: 32 (4.8%) in piglets production farms, 88 (13.8%) in pig finishing farms (in both cases mainly in feed, floor and feces), and 40 (14.8%) in slaughterhouse (mainly in feces, jowls and mesenteric lymph nodes). Among the 210 *Salmonella* spp. isolates, *Salmonella* Typhimurium was the most prevalent (101). PFGE allowed the identification of a continuous entrance of novel *Salmonella* spp. pulsotypes in this food chain, as well as the feed and feces as relevant sources of contamination.

Significance: *Salmonella* spp. was confirmed as a relevant foodborne pathogen in the pork production chain. The identification of the exact contamination routes was important to lead proper procedures to control *Salmonella* spp. in this food chain.

P2-50 Isolation and Comparison of *Escherichia coli* and *Enterococcus* spp. from Two Poultry Management Systems

SHIVARAMU KEELARA[1], Wenhua Liu[2], Rebecca Jones[1], Megan.E. Jacob[1], Anna.T. Rogers[1], Michael.P Martin[1] and Paula.J. Fedorka-Cray[1]

[1]Department of Population Health and Pathobiology, CVM, NCSU, Raleigh, NC, [2]Qingdao Agricultural University, College of Animal Science and Veterinary Medicine, Qingdao, China

Introduction: *Enterococcus* spp. and *Escherichia coli* are commensal bacteria of humans and animals, including poultry that can cause disease under certain conditions. The dissemination/transmission of microorganisms within flocks of birds depends upon several factors including housing, management, and biosecurity. There is a paucity of information describing microbiota of birds housed in different environments.

Purpose: The purpose was to determine differences in *E. coli* and *Enterococcus* spp. prevalence in commercial broiler chickens in conventional housing versus those housed at the Teaching Animal Unit (TAU) at NCSU.

Methods: Day-old chicks from the same hatchery and parent flock (hatched on the same day) were placed in an offsite conventional house (*n*=19,600; sister flock (SF)) and the TAU (*n*=4,000). Ceca (*n*=6) and spleen (*n*=6) were collected weekly from both systems until processing (eight weeks). A loop-full of macerated spleen and cecal contents were plated onto m*Enterococcus* and MacConkey agars for isolation of *Enterococcus* and *E. coli,*

respectively. Presumptive positive isolates were selected phenotypically and speciated/confirmed using MALDI-TOF mass spectrometry. A maximum of eight isolates per sample were selected. Significance was determined using the student t-test ($P<0.05$).

Results: A total of 880 *E. coli* and 800 *Enterococcus* isolates were selected from all samples. Collectively, *Enterococcus durans* (25.8%) was observed more often than *Enterococcus faecalis* (20.8%), *Enterococcus hirae* (19.1%), and *Enterococcus faecium* (2.3%). *Enterococcus* prevalence was higher in ceca from TAU (46 of 48; 95.8%) versus SF (41 of 48; 85.4%). However, a significant difference ($P< 0.04$) was observed from the spleen of SF (5 of 48; 10.4%) versus TAU birds (1 of 48; 2%). *Escherichia coli* was recovered from all ceca (100%); no significant difference was observed in *E.coli* from spleen (TAU (12.5%); SF (14.5%)).

Significance: Differences in recovery of *Enterococcus* spp. from spleen versus ceca was unexpected and warrants further investigation. This observation may be useful for developing mitigation strategies.

P2-51 *Arcobacter* Isolation from Minced Beef Samples in Costa Rica

MARIA LAURA ARIAS[1], Mauricio Redondo[1], Oscar Cordoba[1], Evelyn Carolina Chaves Ulate[1] and Eduardo Castro-Arias[2]

[1]Universidad de Costa Rica, San Jose, Costa Rica, [2]Universidad de Ciencias Medicas, San Jose, Costa Rica

Introduction: The presence of *Arcobacter* spp. in minced meat (including beef) samples has been well documented in different countries with varying frequencies. Nevertheless, the only Latin American country reporting this bacterium in minced beef samples is Mexico, with a 28.8% frequency in 2003. Previous studies in Costa Rica have demonstrated the presence of *Arcobacter* species in samples taken from the poultry production chain, but still there are no studies performed in bovine meat.

Purpose: The aim of this study was to determine the frequency of this bacterium in 120 samples of minced beef acquired from the Central Valley region of Costa Rica, as well as to describe the antibiotic sensitivity pattern of the isolates obtained.

Methods: A total of 120 samples of minced beef were analyzed Isolation was performed using membrane filtration methodology. Genera and species were classified using PCR methodologies. The agar plate dilution method was used according to the Clinical and Laboratory Standards Institute Guidelines to determine the antibiotic resistance profile

Results: A total of 75 different *Arcobacter* strains were isolated from minced beef samples for a final frequency of 48.3%. After species PCR identification, the strains were classified as *Arcobacter butzleri* (37.3%)*, Arcobacter cibarius* (14.7%), *Arcobacter thereius* (12%), and *Arcobacter* spp. (36%). All samples were sensitive to gentamicin, but were resistant to ampicillin, levofloxacin, nalidixic acid, and ciprofloxacin.

Significance: The results obtained in this study show that the frequency of isolation of *Arcobacter* in minced beef samples is high and there is a high resistance rate for common use antibiotics. This suggests that *Arcobacter* represents a health risk for Costa Rica and control measures should be developed in order to decrease its potential impact.

P2-52 Prevalence and Serotyping of *Salmonella* Isolated from Fresh Ground Meats Obtained during a Year-long Surveillance Study of Retail Meat Samples Collected in Eastern South Dakota

ALAN ERICKSON, Debra Murray, Laura Ruesch, Milton Thomas, Zachary Lau and Joy Scaria

South Dakota State University, Brookings, SD

Introduction: *Salmonella* is one of the most common foodborne pathogens found in fresh retail meat products.

Purpose: The purpose of the current study was to determine the prevalence and serotype of *Salmonella* in common types of fresh ground meat available to consumers in regional grocery stores in eastern South Dakota.

Methods: Samples of fresh ground turkey, ground chicken, and ground pork were purchased from grocery stores one time per week for one year. The number of samples of each ground meat purchased each week was dependent on number of different products of each fresh ground meat that were available in the stores. The ground meat samples were tested for the presence of *Salmonella* using the USDA MLG method with BAX PCR as the primary screening assay. Once identified as a positive sample by BAX PCR, *Salmonella* were isolated using standard USDA MLG methods. The identity of the isolated bacteria as *Salmonella* was verified by MALDI-TOF MS biotyping. An Illumina MiSeq genome sequencer was then used to determine the serotypes of the isolates.

Results: For ground turkey, 1.4% (2 of 136) of the samples tested positive for *Salmonella*. For ground chicken, 11% (6 of 55) of the samples tested positive for *Salmonella* with the most common serotype (5 of 6 isolates) being Enteriditis. For ground pork, 37% (49 of 133) of the samples tested positive for *Salmonella.* Twenty different serotypes of *Salmonella* were detected among the ground pork isolates with the most common serotypes being the potential monophasic variant of Typhimurium (10%), Uganda (10%), Anatum (8%), Derby (8%), Infantis (6%), Johannesburg (6%) and London (6%).

Significance: Even though the sample size of this study was relatively small, the results of the study clearly show that retail fresh ground meats continue to be a potential source of *Salmonella* infections.

P2-53 Phage-based Treatment as an Environmental Control Strategy for *Listeria* spp. in a Meat Processing Facility

AJITA SUNDARRAM, Shelby Meyer, Paul Ebner and Haley Oliver

Purdue University, West Lafayette, IN

❖ ***Developing Scientist Competitor***

Introduction: Environmental *Listeria* spp. contamination can subsequently result in contaminated food products. Listeriophages are being studied extensively as a control strategy for *Listeria monocytogenes* and other *Listeria* spp. due to their specificity.

Purpose: The purpose of this study was to evaluate efficacy of a listeriophage cocktail as a *Listeria* spp. control strategy for nonfood contact surfaces (NFCS) in a meat processing facility.

Methods: Initial prevalence, persistence, and load of *Listeria* spp. was determined for 30 floor-wall (FW) junctions and 29 drain sampling sites, once weekly for three weeks, in a meat processing facility, using a modified FDA BAM protocol and standard PFGE typing. Positive sites were treated with listeriophage cocktail in liquid (1 ml/ft^2 [10^9 pfu/ml]), three times at 12 h intervals. Treated and untreated sites were again sampled for *Listeria* spp. and concentrations were compared to pretreatment levels. Lytic capacity of an isolate from each postivie site was determined by standard spot assay.

Results: A total of 15, 21, and 14 samples (among 59 sites) were positive for *Listeria* spp. at weeks one, two, and three, respectively. PFGE analyses identified 38 strains. Twenty-three sites that were positive at least once were treated with listeriophage. Twelve sites remained positive after treatment; *Listeria* spp. were numerically reduced in four of 12 sites by an average of 2.1 log CFU/sponge. Among five PFGE types that were recovered more than once, PUL_0012-PUL_0045 was recovered, consecutively, in week three and four from the same drain. A total of 26% isolates (six of 23) showed

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low susceptibility (average lytic score zero to one) to listeriophage, 43% (10 of 23) showed moderate lysis (score one to two), and 31% (seven of 23) isolates showed confluent lysis (score two to three) across all temperatures and listeriophage concentrations tested.

Significance: Reductions in detected *Listeria* spp., postapplication of listeriophage, offers preliminary evidence that phages may be an effective *Listeria* spp. control strategy for nonfood contact surfaces.

P2-54 Prevalence and Characterization of Antimicrobial-resistant *Campylobacter* Isolated from Eggshells in Different Commercial Laying Hen Housing Systems

ESTEFANIA NOVOA RAMA[1], Matthew Bailey[1], Deana Jones[2], Richard Gast[3], Kenneth Anderson[4], Jagpinder Brar[1], Rhonda Taylor[1], Haley Oliver[1] and Manpreet Singh[5]

[1]Purdue University, West Lafayette, IN, [2]U.S. Department of Agriculture–ARS, Athens, GA, [3]USDA/U.S. National Poultry Research Center, Athens, GA, [4]North Carolina State University, Prestage Department of Poultry Science, Raleigh, NC, [5]University of Georgia, Athens, GA

❖ ***Developing Scientist Competitor***

Introduction: Antimicrobial resistant *Campylobacter* is one of the major threats to public health in the United States, with 310,000 hospitalizations reported by the CDC annually. *Campylobacter* related illnesses have an associated annual cost of $1.9 billion, and over half is due to the neurological disease known as Guillain-Barré syndrome. High prevalence of *Campylobacter* in the internal organs of laying hens has been reported in recent years, thus, concerns with egg safety are on the rise.

Purpose: The aim of this study was to evaluate the effect of different layer housing systems on the prevalence, persistence and antimicrobial resistance of *Campylobacter* isolated from commercial laying hens.

Methods: Over 10 months, 355 samples were collected from five different housing types. A total of 123 isolates recovered from environmental and eggshell samples were presumptively identified as *Campylobacter* spp. by serological tests. Subsequent biochemical identification and real-time PCR assays were performed for confirmation of *Campylobacter*. Antimicrobial resistance of *Campylobacter* was determined using the National Antimicrobial Resistance Monitoring System (NARMS) protocol and genetic variability was assessed by PFGE.

Results: Following PCR, 58% of isolates were confirmed as *Campylobacter* spp., with 91.7% being *Campylobacter jejuni* and 8.3% being *Campylobacter coli*. Samples taken from conventional cages had the highest *Campylobacter* prevalence (23.21%) while those recovered from enrichable cages had the lowest (13.33%). Results demonstrated a high resistance to tetracycline (58%) followed by resistance to nalidixic acid (1.4%); both important antimicrobials for human medicine. The PFGE fingerprinting of *Campylobacter* isolates showed nine types with one leading PFGE type comprising 39% of isolates. Results showed no correlation ($P>0.05$) between *Campylobacter* prevalence and antimicrobial resistance among the housing types studied.

Significance: Findings from this study are concerning and illustrate the need for appropriate interventions to prevent drug-resistant *Campylobacter* spread among laying hens.

P2-55 Isolation and Characterization of Shiga Toxin-producing *Escherichia coli* in Ground Beef from Santiago, Chile

Daniel Rivera, Maria Fernanda Jimenez, Leonela Diaz, Paola Antivero, Paola Navarrete, Angelica Reyes-Jara and **MAGALY TORO**

INTA, University of Chile, Santiago, Chile

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is a pathogen causing sporadic diarrhea, foodborne outbreaks and complications such as hemorrhagic colitis and hemolytic uremic syndrome. The disease is associated with eating or manipulating raw or insufficiently cooked ground beef. In Chile, we lack information on isolation rates and characteristics of STEC present in ground beef.

Purpose: To isolate and characterize STEC from ground beef obtained in Santiago, Chile.

Methods: We collected 430 ground beef samples from retail butcher shops and grocery stores in Santiago, Chile in 2016. After enrichment, samples were screened for Shigatoxin genes (*stx1* and *stx2)*. Isolated colonies were confirmed as STEC through PCR (*E. coli, stx_1, stx_2*), and biochemical characteristics (hemolysin and β-glucoronidase production, tellurite resistance, sorbitol fermentation) were investigated. Also, virulence genes for intimin (*eae*) and hemolysin (*hlyA*) were detected through PCR, besides serogroup identification for O26, O45, O103, O111, O121, O145 (big six) and O157.

Results: STEC was detected in 9.3% (40 of 430) of samples, and 65 STEC isolates were obtained. Most of the positive samples (55%; 18 of 40) were obtained from butcher shops. Isolates carried only stx_1 (22%), only stx_2, (56%) or both Shigatoxin genes (22%). *The hlyA* gene was detected in 29% of isolates, while *eae* was not detected. None of the isolates was identified as serogroup O157 or big six. Almost all isolates (98%) fermented sorbitol and produced β-glucoronidase, and 70% produced α-hemolisyn. Conversely, only 4% were resistant to tellurite.

Significance: Ground beef being sold in Santiago, Chile carries STEC and may represent a risk for consumers. More studies are required to investigate the battery of virulence determinants that may be present in these bacterial population and to assess pathogenic potential. Isolation medium containing potassium tellurite may not be suitable to recover all STEC from food samples since only a few isolates were resistant to the agent.

P2-56 Relatedness of Amylase-producing, Endospore-forming Bacteria from the Alimentary Tract of Commercially Processed Broilers

ARTHUR HINTON, JR. and Kimberly Ingram

U.S. Department of Agriculture-ARS, USNPRC, Athens, GA

Introduction: Competitive exclusion (CE) by bacteria from adult poultry reduces colonization of young chicks by *Salmonella*. CE might include the ability of these bacteria to breakdown complex carbohydrates to produce metabolites that inhibit *Salmonella* growth.

Purpose: To isolate amylase producing, endospore-forming bacteria from poultry and determine the degree of relatedness between isolates.

Methods: Bacteriological media was inoculated with cecal and crop contents from broiler carcasses and incubated at 35°C for 48 h. The incubated media was heated at 80°C for 10 min, plated on Bacillus Agar, and incubated at 30°C for 24 h. Amylase production was determined by growing the bacteria on starch agar, flooding the agar with iodine, and observing for clear zones around bacterial growth. Amylase-producing bacteria were identified using the Biolog Bacterial Identification System, and endospore production was confirmed by phase microscopy. The cellular fatty acid profile of the isolates was determined using the MIDI Sherlock Microbial ID System. The dendrogram program of the Sherlock System was used to determine the degree of relatedness between the isolates.

Results: The isolates were identified as *Bacillus mycoides, Bacillus pseudomycoides*, *Bacillus thuringiensis, Bacillus pumilus*, *Bacillus niabensis*, *Paenibacillus provencensis*, *Paenibacillus borealis*, and *Paenibacillus tarimensis* by the Biolog System. Dendrograms produced by the Sherlock System grouped these isolates into three species based on Euclidean Distances of 10 or less: 1) *B. mycoides, B. pseudomycoides*, and *B. thuringiensis* grouped as *Bacillus cereus*; 2) *P. provencensis*, *P. borealis*, *P. tarimensis*, and *B. niabensis* were grouped as a species not in Sherlock's library; and 3) *B. pumilus* alone.

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Significance: The degree of relatedness of the isolates provides information that can be used to characterize bacteria that may play a role in CE. These findings will be useful in selecting isolates for inclusion in defined probiotic cultures used to reduce colonization of poultry by *Salmonella.*

P2-57 Multilevel Evaluation of Preharvest Interventions to Reduce *Salmonella* spp. in Broiler Farms Using a Ranking Matrix

FRANCESCA MARIE CONTADINI[1] and Fernando Sampedro[2]

[1]*University of Minnesota, St Paul, MN,* [2]*University of Minnesota, College of Veterinary Medicine, St. Paul, MN*

Introduction: Presence of *Salmonella* spp. in the food chain represents an important threat to public health. Mitigation steps are taken to reduce contamination in poultry meat during processing. Preharvest interventions are, however, often overlooked as an important opportunity for further prevention. A holistic approach to evaluate *Salmonella* spp. preharvest interventions demands a multilevel approach by considering several dimensions (scientific, economic, regulatory, and consumer dimensions) and sectors (government, academia, and industry).

Purpose: This study was conducted to evaluate the relative effectiveness of *Salmonella* spp. preharvest interventions in broiler farms using a multilevel approach and to provide broiler producers with a prioritized list of the most feasible interventions.

Methods: A literature review of interventions aimed at preventing *Salmonella* spp. in broiler production was performed. Interventions were included in the analysis if they met the following criteria: in vivo trials, administration of challenge strain, and observed significant reduction of *Salmonella* spp. A ranking matrix and a numerical score was then developed to prioritize the interventions based on the level of *Salmonella* spp. reduction and public health relevance, cost-effectiveness, implementation feasibility, regulatory status, and consumer acceptance.

Results: Research into consumer acceptance resulted in ranking scores assigned to interventions placed into two main categories: vaccination, with a score of zero as there is no reported impact, and probiotic feeding interventions, with a score of +1, as consumers associate positive connotations to the intervention. The scientific literature review returned 81 interventions that fit our criteria, divided into four main categories: competitive exclusion, vaccination, vaccination and competitive exclusion, and bacteriophage. A ranking matrix was applied considering reduction of *Salmonella* spp., trial length, number of birds tested, and type of *Salmonella*.

Significance: Providing producers with tools that have a multilevel approach will improve the adoption of new preharvest interventions aimed at enhancing food safety and further collaboration with academia and government.

P2-58 Intestinal Diseases Aggravate *Campylobacter jejuni* Infection Potential in Broilers

HONG WANG, Juan D.L. Cardenas, Guillermo Tellez, Billy Hargis and Xiaolun Sun

University of Arkansas, Fayetteville, AR

Introduction: *Campylobacter jejuni* is one of the prevalent foodborne pathogens because of pervasive *Campylobacter* intestinal colonization and chicken meat contamination. The intestinal colonization level of *C. jejuni* drives chicken carcass contamination. Notably, the practice of antimicrobial free feeding regiments has exacerbated intestinal diseases such as coccidiosis and necrotic enteritis (NE) in chickens and increased foodborne disease incidences. However, how intestinal diseases affect intestinal *C. jejuni* remains elusive**.**

Purpose: In this study, we hypothesize that intestinal diseases aggravate *C. jejuni* colonization in live birds.

Methods: Broilers were challenged with parasite *Eimeria maxim*a at 14 days of age. The birds were then challenged with *Clostridium perfringens* five days later to induce NE. After additional four days, small intestinal tissues were collected for histopathology and molecular assessments.

Results: We found that *E. maxima* induced intestinal inflammation shown as mass immune cell infiltration into lamina propria, swelling and shortening villi, and crypt elongation compared to unchallenged birds. Interestingly, subsequent *C. perfringens* challenge exacerbated intestinal inflammation with additional severe histopathology shown as bleeding, peeled villus epithelial line, blunted villi, and muscularis externa fibroblast nucleus disarray. Molecular biology examination by real time PCR revealed that coccidiosis induced elevated inflammatory mediators *Infγ* and *Il23* mRNA accumulation. NE further elevated inflammatory mediators *Il1β* and *Tnfα* mRNA expression. Importantly, coccidiosis and NE increased *C. jejuni* 16S RNA accumulation by 11- and 51-folds, respectively, compared to uninfected birds. Altogether, these results suggest that NE aggravates coccidiosis-induced intestinal inflammation and both intestinal diseases increase intestinal *C. jejuni* colonization, which potentiates carcass contamination and campylobacteriosis incidences.

Significance: We found that chicken flocks suffering from intestinal diseases pose a higher risk of *C. jejuni* contamination, hence the preventing and treatment of chicken intestinal diseases will benefit not only reducing foodborne disease incidences but also improving chicken productivity.

P2-59 The Prevalence of *Salmonella* in Organically Produced Chicken Meat Parts

HUSNU SAHAN GURAN and Resat Ciftci

Dicle University, Department of Food Hygiene and Technology, Diyarbakir, Turkey

Introduction: *Salmonella* infections are still one of the most common foodborne bacterial illnesses in developing and developed countries. Especially, poultry meat and meat products are often responsible for *Salmonella* outbreaks in the world.

Purpose: Retail is one of the most critical points to consider for consumer safety before foodstuff reaches the consumer. The aim of this study to determine the prevalence of *Salmonella* in organic frozen chicken meat parts at the retail level.

Methods: A total of 88 organic frozen chicken meat parts (breast, leg quarter and drumstick) were collected from gross markets in Diyarbakir, Turkey. The chicken parts were purchased from the markets in original packages. The parts were evaluated for the presence of *Salmonella* using standard selective enrichment, selective plating and biochemical methods. Presumptive *Salmonella* colonies were confirmed using latex agglutination test.

Results: The prevalence of *Salmonella* was found to be 19.3% in all the analyzed samples. *Salmonella* prevalence in breast and drumstick samples was detected in 28.1% and 22.5%, respectively. None of the leg quarter samples was positive for *Salmonella*.

Significance: The results of this preliminary study showed that frozen chicken meat parts produced organically can be contaminated with *Salmonella*.

P2-60 Spectroscopic Analysis of Meat: Detection of Species and Adulteration

ISMAIL HAKKI BOYACI[1], Gonca Bilge[2] and Banu Sezer[1]

[1]Hacettepe University, Ankara, Turkey, [2]Nanosens A.Ş, Ankara, Turkey

Introduction: In the food industry from the production to consumption, food safety and food quality are important parameters for human health and quality life. Meat is one of the most consumed food product with its high nutritional value. However, availability of meat products is limited due to its high prices. Therefore, meat adulteration is wide spread in meat industry. For this reason, producers have an attention on rapid and reliable, eco-friendly technologies to follow the food processes.

Purpose: In this study potential of Laser Induced Breakdown Spectroscopy (LIBS) and Raman Spectroscopy on determination of meat species and detection of meat adulteration was exhibited.

Methods: In this study chicken, pork and beef meats were discriminated by LIBS and chemometric methods according to mineral component differences. In another application, extracted fat samples that were obtained from cattle, sheep, pig, fish, poultry, goat and buffalo and their salami products were examized by using Raman spectroscopy.

Results: The obtained LIBS spectra of pork, beef and chicken samples were qualitatively discriminated with principal component analysis (PCA) method with 83.37% ratio. Pork-beef and chicken-beef meat mixtures were also analysed with partial least square (PLS) method quantitatively. Determination coefficient (R^2) and limit of detection (LOD) values were 0.994 and 4.4% for pork adulterated beef, and 0.999 and 2.0% for chicken adulterated beef, respectively. The collected Raman data were analyzed with a four-stage PCA method. Seven meat species and their salami products were successfully differentiated from each other according to their origin. According to the results the potential of both LIBS and Raman systems for routine analysis of meat samples' quality is high compared to other methods because these methods are rapid, in situ and accurate.

Significance: The most applied meat adulteration is addition of cheaper meat species to costlier meat species. Consumers must be protected from these adulterations because of economical loses, health implications and religious beliefs.

P2-61 Inactivation of Carbapenem-resistant *Enterobacteriaceae* and *Staphylococcus aureus* by Disinfectants Delivered as a Fog and Vapor

Janak Khatiwada[1], **SHURRITA DAVIS**[2], Leonard Williams[3], Maria Sierra[2] and Dustin Smith[3]

[1]Noth Carolina A&T State University, Kannapolis, NC, [2]North Carolina A&T State University, Kannapolis, NC, [3]North Carolina A&T State University-Center of Postharvest Technologies (CEPHT), Kannapolis, NC

Introduction: Carbapenem-resistant *Enterobacteriaceae* (CRE) are a family of bacteria that are difficult to treat because they have high levels of resistance to third generation antibiotic due to production of extended spectrum beta-lactamase. Certain types of staphylococci bacteria resistant to some antibiotics caused methicillin-resistant *Staphylococcus aureus* (MRSA) infection. Multi drugs resistant CRE and MRSA bacteria are associated with most common etiologic agents that cause infection in hospitals and other facilities.

Purpose: Thus the purpose of this study was to evaluate the efficiency of two disinfectants Vital Oxide and hydrogen peroxide delivered as fog and vapor form against the *Klebsiella pneumoniae, Escherichia coli*, and *S. aureus* on stainless steel contact surface exposes for 10, 15, and 20 minutes.

Methods: The bacterium were dried on stainless steel coupons and placed in three different locations in lab. Two different room decontamination machines were use to deliver the disinfectants for 10, 15, and 20 minutes. After the exposure, stainless steel coupons were collected and immersed in three ml of saline, 20 µl of saline were sampled with bacteria and plated, allow to dry, incubated over night and colonies were counted. Efficacy of disinfectant were calculated and compared to controls by Tukey's studentized range test.

Results: Outcome of this experiment suggest that efficacy of disinfectants depends on the time, even though Vital Oxide was significantly ($P<0.05$) more effective (1.15±0.03) in reducing *K. pneumonia* than *S. aureus* with 10 minute of exposure compared to the other treatments and controls.

Significance: These data suggest that Vital Oxide showed more antibacterial activities against the CRE than MRSA, which can be used as a alternative chemicals to decontamination of equipment and health care facilities.

P2-62 Detection of *stx1* and *stx2* Genes in *Escherichia coli* Isolated from Minas Frescal Cheese Processing Plants in São Paulo State, Brazil

Giovana Rueda Barboza[1], Giovana Verginia Barancelli[2], Marjory Xavier Rodrigues[3], Rodrigo T. Hernandes[4], Raiza Iacuzio[1] and **NATHÁLIA CRISTINA CIRONE SILVA**[1]

[1]University of Campinas, Department of Food Science, Faculty of Food Engineering (FEA), Campinas, Brazil, [2]Universidade de São Paulo, Piracicaba, Brazil, [3]Department of Food Engineering, Federal University of Southern Frontier (UFFS), Laranjeiras do Sul, Brazil, [4]Universidade Estadual Paulista, Bioscience Institute, Botucatu, Brazil

Introduction: Diarrheagenic *Escherichia coli* has many virulence factors and some of them can be involved in foodborne disease. In addition, antibiotic resistant strains of *E. coli* are a known public health problem. The indiscriminate use of antimicrobials in livestock is a concern and it has been indicated as responsible for an increase in resistant bacteria.

Purpose: The aim of this study was to detect the occurrence of *stx*1 and *stx*2 genes in *E. coli* isolated from Minas frescal cheese processing plants located in São Paulo State, Brazil, and to verify the phenotypic antibiotic resistance profiles.

Methods: Twelve samples were collected in six processing cheese plants. During each visit raw milk, pasteurized milk, cheese, environment, equipments, utensils, and surface samples were collected. MacConkey agar and eosin methylene blue (EMB) agar were used for bacterial isolation. The confirmation of species was performed using biochemical tests. Detection of *stx*1 and *stx*2 was carried out by polymerase chain reaction (PCR). Antibiotic susceptibility testing was performed by disk-diffusion method using ciprofloxacin, cefoxitin, ceftazidime, chloramphenicol, cefotaxime, trimetopim, tetracycline, streptomycin, nalidixic acid, imipenem, tobramycin, gentamicin, amoxicillin-clavulanic acid, fosfomycin, kanamycin, trimethoprim sulfamethoxazole, rifampicin, aztreonam, and cefepime disks.

Results: Strains were isolated from raw milk, pasteurized milk, cheese, table, floor, coagulation tank, and drain samples. Fourteen *E. coli* strains carrying the *stx1* gene and ten carrying the *stx*2 gene were identified. The presence of *E. coli* carrying the *stx1* gene in pasteurized milk was of note. Regarding *stx1* and *stx2* gene positive strains: one was resistant to rifampicin and the other strains were sensitive to the antibiotics tested.

Significance: The results showed that the production of Minas frescal cheese needs to be studied more in regard to food safety because the consumption of this dairy product represents a possible risk to consumers. Furthermore, the producers need to improve the hygiene conditions along the processing chain.

P2-63 Methicillin-resistant *Staphylococcus aureus* Isolated from the Organic and Conventional Cheese Processing Chains in São Paulo State, Brazil

Talita Junia Silva Cândido[1], Anderson Clayton da Silva[1], Marjory Xavier Rodrigues[2], Vera Lucia Mores Rall[3], Maristela da Silva do Nascimento[4] and **NATHÁLIA CRISTINA CIRONE SILVA**[1]

[1]University of Campinas, Department of Food Science, Faculty of Food Engineering (FEA), Campinas, Brazil, [2]Department of Food Engineering, Federal University of Southern Frontier (UFFS), Laranjeiras do Sul, Brazil, [3]Universidade Estadual Paulista, Bioscience Institute, Botucatu, Brazil, [4]University of Campinas (UNICAMP), Department of Food Technology, Faculty of Food Engineering (FEA), Campinas, Brazil

Introduction: *Staphylococcus aureus* may be a source of food poisoning. *Staphylococcus aureus* has the ability to develop antibiotics resistance, becoming more difficult to treat people infected with this microorganism. In recent years, cases of methicillin-resistant *S. aureus* (MRSA) have been reported in food and food producing animals.

Purpose: The objective of this research was to isolate *S. aureus* from Minas frescal cheese processing plants in organic and conventional production and to analyze possible resistance to antibiotics.

Methods: Samples from cheese, raw milk, and the processing chain environment (floor, surfaces, foodhandlers) from three organic and three conventional dairies were collected. For detection of coagulase-positive staphylococci (CPS), the samples were incubated on Baird Parker agar (35°C/48 h), followed by performance of catalase, Gram stain, and coagulase tests.

Results: One hundred fifty-one CPS were isolated; 83 from organic and 68 from conventional dairies. The PCR species confirmation identified 73 *S. aureus;* 34 from organic and 39 from conventional dairies. The antibiogram was performed for 39 strains identified as *S. aureus*, 31 strains from organic dairy products, and eight strains from conventional dairy products. Fifteen strains were sensitive to all the antibiotics. All of the strains were sensitive to the chloramphenicol, seven strains showed simultaneous resistance to oxacillin and cefoxitin (four organic and three conventional), suggesting that they are MRSA. Resistance was observed in organic dairies to penicillin (*n*=15), gentamicin (*n*=3), tobramycin and erythromycin (*n*=5), tetracycline (*n*=2), and clindamycin (*n*=6). In conventional dairies resistance to penicillin (*n*=4), tobramycin and erythromycin (*n*=1), tetracycline (*n*=1), and clindamycin (*n*=1).

Significance: The data collected in the research presented showed that more than half of the strains of *S. aureus* were resistance to some type of antibiotic, with food being a carrier of resistant bacteria capable of causing disease in consumers.

P2-64 Characterization of Microbiota of Oyster Larvae and Tank Water from an Aquaculture System with High and Low Larval Survival Rates

ANDREA OTTESEN[1], Padmini Ramachandran[1], Elizabeth Reed[1], Angelo DePaola[2] and Scott Rikard[3]

[1]U.S. Food and Drug Administration, College Park, MD, [2]U.S. Food and Drug Administration, Dauphin Island, AL, [3]Auburn University, Mobile, AL

Introduction: Aquaculture plays an increasingly important role in the growing demand for seafood. Oyster hatcheries are an integral component of oyster farming as they provide a source of healthy larvae that can be seeded in designated waters. Larvae from certain tanks in an established aquaculture enterprise were dying from an unknown etiologic agent. A metagenomic approach was used to examine tanks (water and larvae) with high and low survival rates to evaluate the epidemiological efficacy of this approach. Few diseases of oysters are currently well characterized genomically.

Purpose: The goals of this study were to: (i) describe and contrast the microbiome of tank water and oyster larvae from tanks with high (100%) and low (70%) survival rates and (ii) identify the causal agent of oyster larvae mortality in an aquaculture system.

Methods: DNA from source water, tank water, and larvae from three oyster aquaculture tanks was shotgun sequenced and analyzed using Metaphlan, Cosmos ID, and Platypus Conquistador Bioinformatic pipelines. Tank 1 had a 100% oyster larvae survival rate, tank 2 had a 82% survival rate, and tank 3 had a 70% survival rate of oyster larvae.

Results: Oyster larvae from tanks with high and low survival rates were dominated by the bacterial genus *Ruegeria*. Tank water also supported *Vibrio, Mesoflavibacter,* and *Alteromonas* spp. The most distinctive contrast in high survival and low survival larvae was observed in viral profiles. *Listonella* and V*ibrio* phages dominated larvae from tanks with lower survival rates. These two phages were not observed at all in the majority of healthy larvae samples.

Significance: Phages and viral elements provided the most distinctive and potentially diagnostic signatures in this study. The lack of *Listonella* and *Vibrio* phage in the larvae from the 100% survival tank suggests that these bacterium may be playing a role in the mortality of the oyster larvae.

P2-65 Association between Shiga Toxin-producing *Escherichia coli* Prevalence and Biosecurity Measures on Diversified California Farms

LAURA PATTERSON[1], Nora Navarro-Gonzalez[2], Peiman Aminabadi[2], Michele Jay-Russell[2] and Alda Pires[1]

[1]University of California-Davis, Department of Population Health & Reproduction, Davis, CA, [2]University of California-Davis, Western Center for Food Safety, Davis, CA

❖ ***Developing Scientist Competitor***

Introduction: The increasing popularity of small to medium-sized diversified farms reflects growing consumer interest in sustainable agriculture and locally-produced food. Diversified farms are defined as those farms that integrate livestock and produce or raise multiple livestock species. However, livestock may harbor foodborne pathogens that can cause severe human infection, like Shiga toxin-producing *Escherichia coli* (STEC).

Purpose: The aim of this study was to assess the association between biosecurity practices and the prevalence of STEC in livestock raised on diversified farms.

Methods: This study analyzes a subset of 14 farms that were part of a larger year-long cross-sectional study conducted from 2015 to 2016. Cattle, swine, and small ruminant fecal samples (*n*=503) were cultured for STEC and positive isolates were serogrouped. Each farm completed a survey that included questions regarding livestock health, biosecurity, and management practices. An overall biosecurity score was calculated from the farm survey. Based on the median (32 points), the biosecurity score was divided into high (range 32 to 45) or low (range 20 to 31). Logistic models were used to evaluate the association between a high or low biosecurity score and STEC presence, using generalized linear mixed models with farm as a random effect.

Results: STEC was found in 13.72% (69 of 503) of samples and on 57.14% (8 of 14) of farms. Livestock raised on diversified farms with higher levels of biosecurity were less likely to be STEC positive (odds ratio (OR): 0.19; 95% confidence interval (CI): 0.048, 0.76). Other significant factors included raising cattle (OR: 4.06; 95% CI: 1.43, 11.61); farming less than 42 acres (OR: 11.71; 95% CI: 2.43, 56.5); and farming for more than 15 years (OR: 9.97; 95% CI: 1.59, 62.1).

Significance: This study highlighted the need to further investigate potential food safety risks on diversified farms. Identification of strategies to reduce these risks will promote public and livestock health in this emerging agricultural industry.

P2-66 Commonalities of Antimicrobial-resistant Nontyphoidal *Salmonella* among Human and Retail Food Isolates, Tennessee, 2010 through 2013

SAMIR HANNA, John Dunn and Katie Garman
Tennessee Department of Health, Nashville, TN

Introduction: Over 100,000 antimicrobial resistant nontyphoidal *Salmonella*(AR-NTS) infections are reported in the United States, annually. Resistance to clinically important antimicrobials poses public health risks. Food animals are reservoirs of AR NTS that infect humans. The National Antimicrobial Resistance Monitoring System (NARMS) monitors antimicrobial resistance (AR) of enteric bacteria in human, retail meat, and food animals.

Purpose: Review of recent NARMS data in Tennessee to compare AR among NTS isolated from humans and retail meats samples.

Methods: Tennessee submits to NARMS every 20thhuman NTS isolate detected statewide and all NTS isolates from retail meat purchased from four counties. Broth microdilution is performed to determine MIC for 14 antimicrobials. Resistance patterns among human isolates were compared to those in retail meats. Analysis was done using Epi Info 7.

Results: From 2010 through 2013, 179 sporadic NTS human isolates and 76 NTS retail meat isolates were tested. Twenty-two (12%) NTS human isolates and 54 (71%) NTS retail meat isolates demonstrated AR. AR to ≥3 classes was detected in 13 (7%) NTS human isolates, six (18%) chicken breast isolates, and 14 (45%) ground turkey. Four (2%) human isolates were resistant only to ACSSuT; all were serotype *Salmonella* Typhimurium. Resistance to quinolones was detected in two (1%) human isolates; serotypes *Salmonella* Typhimurium and *Salmonella* Litchfield. Only one (0.6%) of NTS human isolates was resistant to ceftriaxone compared to two (6%) and five (16%) from chicken breast and ground turkey isolates, respectively. Ceftriaxone-resistant human, chicken, and turkey isolates were differing serotypes. No retail meat isolates were quinolone resistant.

Significance: AR is a public health concern. In Tennessee, AR-NTS human isolates occurred less commonly than some national estimates, particularly AR to CIAs. Retail meat isolates demonstrated AR more frequently than human NTS with ground turkey isolate AR being more common than chicken. Serotypes varied among AR isolates from all three sources.

P2-67 Integrated Food Safety Centers of Excellence: Increasing State and Local Capacity to Detect and Investigate Foodborne Disease through Peer-to-Peer Support

David Dekevich[1], **YNES R. ORTEGA**[2] and Jamie DeMent[3]
[1]Florida Department of Health, Jacksonville, FL, [2]University of Georgia, Griffin, GA, [3]Florida Department of Health, Tallahassee, FL

Introduction: Foodborne diseases cause approximately 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths annually. Decreased resources have negatively impacted foodborne disease outbreak investigation and response capacity. The Food Safety Modernization Act (FSMA) required that CDC designate Integrated Food Safety Centers of Excellence (CoEs) at state health departments in partnership with academic institutions.

Purpose: This study gathered information to describe how CoEs address capacity limitations by serving as resources for local, state, and federal public health professionals to reduce the burden of foodborn disease.

Methods: Colorado, Florida, Minnesota, New York, Oregon, and Tennessee were chosen as CoEs through a competitive review process. Using their unique health department/academic institution partnerships, CoEs provide peer support by using needs assessment results to develop educational materials, deliver trainings, and provide consultation services. Activities of the CoEs include: strengthening surveillance and outbreak investigation; analyzing the timeliness and effectiveness of responses; training public health staff in proven investigation and surveillance techniques; educating the future food safety workforce; improving capacity of information systems; and evaluating and communicating best practices.

Results: CoEs have developed over 100 online products on a variety of topics that are available for free, immediate use. Collectively, CoE training videos have been viewed over 50,000 times on YouTube. CoEs have provided one-on-one assistance to a number of state and local health departments, including 18 states funded under the CDC OutbreakNet Enhanced Program. Capacity has been created to provide assistance in the form of consultation for long-term projects or more urgent consultation during ongoing outbreaks.

Significance: Since their establishment, CoEs have addressed FSMA requirements by developing online tools and providing guidance and support to other health departments, which has improved foodborn illness surveillance and response capacity across the country. CoEs provide valuable resources and enhance the efficiency of surveillance and investigation activities at no cost to the receiving agency.

P2-68 Whole Genome SNP Analysis of *Salmonella* Enteritidis Strains Isolated between 1968 and 2016 in Brazil

FABIO CAMPIONI[1], George Kastanis[2], Guojie Cao[2], Maria Hoffmann[3], Yan Luo[3], Alzira Maria Morato Bergamini[4], Dalia dos Prazeres Rodrigues[5], Marc Allard[3] and Juliana Pfrimer Falcao[6]
[1]University of São Paulo, School of Pharmaceutical Sciences of Ribeirao Preto, Ribeirao Preto, Brazil, [2]U.S. Food and Drug Administration, Division of Microbiology, Office of Regulatory Science, Center for Food Safety and Nutrition, College Park, MD, [3]U.S. Food and Drug Administration, College Park, MD, [4]Adolfo Lutz Institute, Ribeirao Preto, Brazil, [5]Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [6]University of São Paulo, School of Pharmaceutical Sciences of Ribeirao Preto, Ribeirao Preto, Brazil

Introduction: *Salmonella* Enteritidis emerged after the 1980s as the most common foodborne isolated serovar in several countries. In Brazil the emergence was observed after the 1990s and it is believed that an epidemic and more virulent clone was introduced in the country. This serovar remains as one of the most isolated one from clinical and food sources in Brazil.

Purpose: A comparative whole genome single nucleotide polymorphism (SNP) analysis of *Salmonella* Enteritidis strains isolated in Brazil over a period of 48 years was performed to check the diversity of the strains in the pre- and postpandemic period.

Methods: DNA from 267 *Salmonella* Enteritidis strains isolated from food (85), humans (113), chickens (52) and the farm environment (17) in Brazil between 1968 and 2016 were sequenced using the Illumina Nextseq platform. The sequencing reads were mapped to the reference strain *Salmonella* Enteritidis P125109 to build a SNP matrix and construct the phylogenetic tree. We also included 30 *Salmonella* Enteritidis strains isolated in the United States in the analysis for comparison purposes.

Results: Based on the SNP analysis, 18 of the 26 strains isolated until 1993 clustered together and in a different cluster from the strains isolated between 1994 and 2016. The United States strains clustered together in a separated cluster. We analyzed the genomic diversities of *Salmonella* pathogenicity islands, *Salmonella* genomic islands and other virulence factors.

Significance: The study demonstrates that use of next-generation sequencing identifies stable SNP targets that can be used for differentiating closely related *Salmonella* Enteritidis isolates, providing a better understanding of virulence and evolutionary history. The results suggest a genomic

difference among strains isolated before and after the pandemic in Brazil, which helps to explain the emergence and the outbreak transmission dynamics of *Salmonella* Enteritis in Brazil after the mid-90s.

P2-69 Relationship between Production System or Animal Attributes and the Counts of Pathogenic Shiga Toxin-producing *Escherichia coli* O157, O26, and O111 in Australian Beef Cattle at Slaughter

GLEN MELLOR[1], Narelle Fegan[2], Lesley Duffy[1], Kate McMillan[1], David Jordan[3] and Robert Barlow[1]

[1]CSIRO Agriculture & Food, Brisbane, Australia, [2]CSIRO Agriculture & Food, Werribee, Australia, [3]NSW Department of Primary Industries, Wollongbar, Australia

Introduction: At specific thresholds, the concentration of pathogenic shiga toxin-producing *Escherichia coli* (pSTEC) in cattle feces can have an impact on the likelihood and extent of carcase contamination during processing.

Purpose: This study determined the range of counts of pSTEC serotypes O157, O26, and O111 in the feces of beef cattle at slaughter, which were previously deemed positive for pSTEC and assessed whether a relationship existed between pathogen counts and production systems or animal attributes.

Methods: The pSTEC counts were determined for cattle fecal samples (*n*=111) using a combined most probable number and real-time PCR approach. Analysis was conducted by comparing animal class (veal, dairy, and beef), carcass weight, plant processing volume, or production system (grass, grain, and dairy) with the frequency of counts, which fell into a range that was either greater or less than a single log MPN g^{-1} value. The values used were one, two, three, four, and five log MPN g^{-1}. Comparisons were performed in contingency tables using chi-squared or Fisher's exact tests and relationships were deemed significant if $P<0.01$.

Results: Counts of pSTEC ranged from <-0.52 to 6.89 log MPN g^{-1} feces. At a *P* value of <0.01, the only association observed was in production systems where pSTEC counts of <one log MPN g^{-1} were more frequent in grass-fed (62.5% of 48) than in grain-fed animals (7.7% of 13). The study found no evidence of a relationship between the frequency of count (at any given log range) and animal class, carcass weight, or plant processing volume.

Significance: Previous studies suggest that an increased risk of pathogen transmission during processing is most likely to occur when the concentration of pSTEC exceed three log cfu g^{-1} feces. At this threshold, none of the factors investigated would indicate an increased risk of carcass contamination during processing.

P2-70 Milk Contamination and Prevalence of *Escherichia coli* O157:H7 in Kwara State, Nigeria

IBRAHEEM GHALI-MOHAMMED[1], Ismail Odetokun[1], Ibrahim Raufu[2] and Victoria Adetunji[3]

[1]University of Ilorin, Department of Veterinary Public Health and Preventive Medicine, Ilorin, Nigeria, [2]University of Ilorin, Ilorin, Nigeria, [3]University of Ibadan, Department of Veterinary Public Health and Preventive Medicine, Ibadan, Nigeria

Introduction: Milk handling and milking practices in the Nigerian informal sector are done commonly without observing hygienic practices of vended cattle milk, and this is a threat to food safety and public health. However, empirical evidence to confirm quality of milk produced by local processors is lacking.

Purpose: A cross-sectional study was carried out to determine the quality and prevalence of *Escherichia coli* O157 in raw cattle milk processed and vended in Kwara state, Nigeria.

Methods: Total aerobic plate count (TAPC), Total coliform count (TCC), Methylene blue reductase test (MBRT), and California mastitis test (CMT) were carried out to determine the quality and wholesomeness of randomly collected raw cattle milk (*n*=552), across various markets (*n*=10) in the major zones (Central, North, and South) of the state. Isolation of *E. coli* was done using standard microbiological procedures while presence of the *E. coli* O157: H7 was confirmed with serology.

Results: Mean TAPC ranged from logcfu 8.5±1.4 to log cfu 11.5±0.6 while all TCC were higher than log cfu five. CMT revealed that 58.5% and 41.5% of milk samples were positive and negative respectively for mastitis causing organisms. The overall prevalence of *E. coli* was 38.3% and 1.3% for *E. coli* O157:H7. The prevalence (43.2%) of isolated *E. coli* was significantly higher ($P<0.05$) in the North than other zones.

Significance: Our study determined the presence of pathogenic *E. coli* and largely poor quality of vended raw milk in the markets. In view of public safety, the need to demonstrate a high level of hygienic practice during milk processing and enlightenment of processors on the need for standard hygiene is required.

P2-71 The Main Source of *Clostridium difficile* in the Community is Nature

CRISTINA RODRIGUEZ[1], Joahn Van broeck[2], Michel Delmée[2] and Georges Daube[1]

[1]University of Liege, Liege, Belgium, [2]Catholic University of Leuven, Microbiology, Brussels, Belgium

Introduction: *Clostridium difficile* is considered the leading cause of antibiotic associated disease worldwide. In the last decade a large number of studies were focused on identifying the main sources of contamination and natural reservoirs in order to elucidate the complete life cycle of the infection. Hospitals environments, food animals, pets and retail foods have been considered as potential vectors. However, the prevalence of *C. difficile* in these types of samples was found to be rather low, suggesting that other contamination routes must exist.

Purpose: This study is one of the few to explore the presence of *C. difficile* in the natural environment, and specifically away from the urban cores.

Methods: Farmlands and surroundings, residential, walking areas, and forests were studied. Samples, including mud, water, grass, roots, and stones, were collected from soils (top soil: organic debris partly decomposed). Sampling was performed from November to December 2016 with an outside temperature ranging between 2 and 8°C. *Clostridium difficile* was isolated from the samples by direct and enrichment broth.

Results: An unexpected, very high prevalence was found in this study. A total of 29 out of 59 samples were positive for the bacterium (50.8%). The viable spore count from soils varied between 50 and 250 cfu/g. Spores were detected more frequently on the roadsides than into the forests and farmlands. These findings may indicate that animals, including dogs, foxes, wild boards and birds among others are responsible of the spread of *C. difficile* spores. Furthermore, the high prevalence found indicates that the bacterium probably forms part of the normal gut microbiota of some animals. Further work should address if the presence of spores in this environment increases in spring and/or summer and the factors associated with this increase.

Significance: Results of this study reveal that humans are continuously exposed to the bacterium by a highly contaminated natural environment and only a disruption in the gut causes the disease.

P2-72 Defining a Core Genome Multilocus Sequence Typing Scheme for the Global Epidemiology of *Vibrio parahaemolyticus* Strains

NARJOL GONZALEZ-ESCALONA[1], Keith A. Jolley[2], Elizabeth Reed[1] and Jaime Martinez-Urtaza[3]

[1]*U.S. Food and Drug Administration, College Park, MD,* [2]*Department of Zoology, University of Oxford, Oxford, United Kingdom,* [3]*University of Bath, Bath, United Kingdom*

Introduction: *Vibrio parahaemolyticus* is an important human foodborne pathogen whose transmission is associated with the consumption of contaminated seafood. A multilocus sequence typing (MLST) database for *V. parahaemolyticus* is available and has been used to identify a large number of clones that have caused severe outbreaks worldwide or recurrent outbreaks in certain regions, or have spread to other regions where they are non-endemic. The current MLST scheme is a powerful tool for inferring the population structure of this pathogen; but it has limited resolution, especially when compared to pulse field gel electrophoresis.

Purpose: Application of whole genome sequencing has become a routine for tracing infections or outbreaks. Core genome MLST (cgMLST) analysis is one of the most straightforward ways to explore complex genomic data in an epidemiological context. Therefore, there is a need to generate a new, portable, standardized, and more advanced system that provides higher resolution among *V. parahaemolyticus* strains using WGS data.

Methods: To establish this cgMLST scheme, we sequenced 92 *V. parahaemolyticus* genomes and used the genome of strain RIMD 2210633, as the reference (total 4,832 genes), to determine which genes were suitable for establishing the *V. parahaemolyticus* cgMLST scheme.

Results: The initial analysis resulted in the identification of 2,254 suitable core genes for use in the scheme. To evaluate the performance of this scheme, we performed a cgMLST analysis of the 92 newly sequenced genomes, plus an additional 142 strains with genomes available at NCBI. The cgMLST scheme distinguished related and unrelated strains, including those with the same sequencing type; clearly showing its enhanced resolution over conventional MLST analysis. This cgMLST also distinguished outbreak-related from unrelated strains within the same sequencing type.

Significance: Application of this cgMLST scheme to *V. parahaemolyticus* strains from different laboratories around the world will facilitate a global picture of the epidemiology, spread, and evolution of this pathogen.

P2-73 Infrastructures, Sanitation, and Management Practices Impact *Listeria monocytogenes* Prevalence in Retail Grocery Produce Environments

TONGYU WU[1], John Burnett[1], Jingjin Wang[1], Susan Hammons[1], Deklin Veenhuizen[1], Manpreet Singh[2] and Haley Oliver[1]

[1]*Purdue University, West Lafayette, IN,* [2]*University of Georgia, Athens, GA*

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Introduction: In an ongoing study by our group, *Listeria monocytogenes* is highly prevalent in some, but not all, retail grocery produce departments. Risk factors contributing to high *L. monocytogenes* prevalence are largely unknown.

Purpose: Our study aimed to determine if facility design and management practices affect *L. monocytogenes* prevalence in retail produce environments, which will subsequently inform intervention strategies to reduce *L. monocytogenes*.

Methods: We designed a comprehensive, 111 question survey to capture facilities, cleaning and sanitation frequencies, employee training, work shift arrangement, equipment usage, etc. Retail produce managers from 30 retail produce departments among seven states in the United States completed the survey in stores conducting monthly *L. monocytogenes* environmental sampling. Linear regression with Pearson correlation, ANOVA, and subsequent Tukey analyses were used to identify significant factors that impact *L. monocytogenes* prevalence (α=0.05).

Results: By Tukey comparison (α=0.05), presence of standing water near misted produce case drain covers (P=0.0125), increased time taken for cleaning after the produce preparing area has closed (P=0.0038), presence of a bottom shelf level that is inaccessible for cleaning, and holding dry produce (P=0.0217) significantly increased *L. monocytogenes* prevalence. Cleaning produce retail case food contact surfaces once every two to four days resulted in higher prevalence compared to once every four hours, once daily, and frequencies less than once per two weeks (P=0.0029). Changing gloves after handling each type of produce (P=0.0114), selecting role models among employees (P=0.0128), being aware of how gloves are managed (P=0.0015), and absence of traffic from the produce area to other departments during work (P=0.0394) strongly reduced prevalence. Counterintuitively, trafficking from deli to produce was associated with lower *L. monocytogenes* prevalence (P=0.0243).

Significance: This was the first study to investigate the impact of facility design and management practices on *L. monocytogenes* prevalence in retail produce environments. Verification of sanitation and management practices will identify additional risk factors.

P2-74 Food Safety Practices at Various Restaurants and Hotels in Lahore, Pakistan

MUHAMMAD SHAHBAZ[1], Muhammad Nasir[2], Zubair Farooq[2] and Muhammad Bilal[3]

[1]*Mawarid Food Company - KSA (Pizzahut, Taco Bell), Riyadh, Saudi Arabia,* [2]*University of Veterinary and Animal Sciences Lahore Pakistan, Lahore, Pakistan,* [3]*University of Agriculture, Faisalabad, Pakistan*

❖ ***Developing Scientist Competitor***

Introduction: Food safety, a rapidly growing and serious public health concern in developing countries, has diverted the focus of food safety experts, policy makers, and legislators to formulate and standardize food safety legislations with proper enforcement to give access to safe, hygienic, and wholesome food.

Purpose: The purpose of study was to assess and evaluate the current food safety practices adopted by various restaurants and the food safety knowledge and awareness among food handlers.

Methods: In the present study a cross sectional survey of 120 restaurants and hotels in nine towns of Lahore, which are under jurisdiction of the Punjab Food Authority, were carried out in collaboration with the Punjab Food Authority.

Results: Results of this study showed that restaurants and hotels have not properly adopted food safety practices. On part of management and personnel, the situation at food establishments is highly pathetic; 36% of restaurants needed immediate improvement, 75% needed major improvement, and only 2% of the food establishments showed good signs of safety practices. The situation of personnel hygiene is also very poor; 17% needed immediate improvement and 26% required major improvement, and none of the restaurants have meet very good criteria. For 80% of restaurants, immediate improvement was required because of maintenance and infrastructure. In response to control of operation, 60% need urgent improvement.

Significance: This study was quite helpful for recognizing restaurants and hotels that need to improve and adopt food safety practices.

P2-75 Multitoxin Production by *Bacillus cereus* and *Staphylococcus aureus* in Co-contaminated Ready-to-Reheat Lasagna as a Function of Heat Treatment, Modified Atmosphere, and Storage Temperature

ANDREJA RAJKOVIC[1], Mirjana Andjelkovic[2] and Mieke Uyttendaele[1]
[1]*Ghent University, Ghent, Belgium,* [2]*Scientific Institute of Public Health, Brussels, Belgium*

Introduction: *Bacillus cereus* and *Staphyloccous aureus* are pathogens with a variety of toxins acting, primarily, as virulence factor. Both pathogens are very frequently isolated from different foods. Not much is known about their behaviour if copresent in the same food.

Purpose: This study assessed cogrowth and cotoxin production of *B. cereus* and *S. aureus* in typical ready-to-reheat foods.

Methods: Three strains of *B. cereus* (two emetic and one diarrheal strain) and two *S. aureus* enterotoxigenic strains were used in for the inoculations. *Bacillus cereus* was inoculated either as spores or vegetative cells. Spores were initially treated with 10 minutes at 70, 80, or 90°C to assess the impact of mild heat treatments. Ready-to-reheat lasagne bolognaise packaged in modified atmosphere was purchased at a local supermarket, inoculated, MAP packed (8% O_2, balanced by N_2) and stored at 12 and 22°C. Microbial and toxin analyses were regularly performed.

Results: At 22°C modified atmosphere did not influence the maximum cell count reached by *S. aureus*, while for *B. cereus* a relatively small difference could be seen. When the experiment was performed with vegetative cells instead of spores of *B. cereus*, a slower initial growth could be seen, but the differences levelled off. The storage day cereulide was detected and the corresponding cereulide concentration were the same. The modified atmosphere at 22°C did not have an influence on the onset of staphylococcal enterotoxin (SE) production or the amount produced. SEs were always produced at a lower cell density than cereulide. At 12°C, a pronounced difference in growth between *B. cereus* and *S. aureus* could be seen. At 12°C only SE were produced under all MAP conditions; CER was never found and HBL and NHE production by *B. cereus* varied in function of MAP.

Significance: Data showed that both *B. cereus* and *S. aureus*, as well as their toxins, can be present in high amounts in the same food.

P2-76 Occurrence of the Principal Mycotoxins in Food and Feed in Serbia from 2004 to 2017

ANDREJA RAJKOVIC[1], Bozidar Udovicki[2] and Nikola Tomic[3]
[1]*Ghent University, Ghent, Belgium,* [2]*University of Belgrade-Faculty of Agriculture, Department of Food Safety and Food Quality Management, Belgrade, Serbia,* [3]*University of Belgrade-Faculty of Agriculture, Belgrade - Zemun, Serbia*

Introduction: This paper represents a review of principal mycotoxins in food and feed in Serbia after the year 2004. As secondary metabolites produced by several genera of fungi, mycotoxins are usually found in agricultural commodities. With an annual production of 7.7 M tons and 2.5 M tons for maize and wheat, respectively, Serbia is one of the prominent grain producers and exporters in Europe. Cereals are the major staple food for the Serbian population, having high social and nutritional relevance and, therefore, represent an important food group.

Purpose: In view of high grain consumption, the presence of mycotoxins entails a high risk for acute and chronic exposure to mycotoxins.

Methods: In silico analysis of data in the scientific literature, datamining in the RASFF database, and official monitoring data were used in this review.

Results: The most striking example are aflatoxins; total prevalence was 54.5% (*n*=3,246) and 32.4% of the samples exceeded the EU limits during this period. Similar results were obtained for T-2/HT-2 (*n*=432), deoxynivalenol (*n*=2,816), fumonisins (*n*=429), zearalenone (*n*=638) and ochratoxin A (*n*=609) with prevalences of 40.5%, 41.9%, 66.4%, 35.1%, and 30.2%, respectively. For these mycotoxins, the EU limits were less frequently exceeded. The increased incidence of mycotoxins in Serbian grain matrices is thought to be caused by more favourable environmental conditions in combination with the use of more sensitive immunosorbent assays and chromatography methods.

Significance: The available information reviewed in this paper, showed low and moderate incidences and prevalences of mycotoxins in food and feed in Serbia; the exceptions being 2012, a drought year, and 2014, a flood year. The number of samples that were above limits set by EU legislation was relatively low. Relatively higher numbers of positive samples, in recent studies, were due to advances in detection techniques. A relatively low number of analyzed samples hampers reliable estimates of mycotoxins prevalence and concentration in certain food and feed commodities.

P2-77 Evaluating the Impact of Cooling Techniques on *Bacillus cereus* Populations in Brown Rice

Lindsay Beardall[1], Paola Paez[2], Randall Phebus[2], Bryan Severns[1], Tracee Watkins[2] and **SARA GRAGG**[1]
[1]*Kansas State University, Olathe, KS,* [2]*Kansas State University, Manhattan, KS*

Introduction: In institutional settings, large quantities of food may be cooked, cooled, and stored for later service. Improper, or slow, cooling has been identified by the United States Food and Drug Administration as a contributing factor in foodborne illness outbreaks. Therefore, validating cooling methods that are feasible and effective at preventing pathogen growth is critical for public health.

Purpose: This study was designed to test the efficacy of cooling technique combinations on controlling *Bacillus cereus* growth within brown rice.

Methods: Brown rice was prepared according to product label instructions and then cooled to 135 to 140°F before inoculation with *B. cereus* (10^4 CFU/g of spores). All pans were stored in a commercial walk-in freezer (-20°C) or placed in ice water baths stored inside a commercial walk-in refrigerator (4°C), either uncovered or covered with one or two layers of aluminum foil. Samples were obtained at 0, 4, 8, 12, and 24 hours, plated onto Mannitol Egg Yolk with Polymyxin B agar, and incubated for 24 to 48 hours to enumerate *B. cereus* populations.

Results: Treatment*time (*P*=0.0026) and product depth*time (*P*=0.0268) were statistically significant for *B. cereus* populations within the brown rice product during cooling. *Bacillus cereus* populations decreased by 0.37 log CFU/g between zero and 24 hours when stored in the freezer, whereas populations decreased by 0.09 log CFU/g between aero and 24 hours when stored in the refrigerator. *Bacillus cereus* populations decreased in both two- and three-inch product depths between zero and 24 hours by 0.21 log CFU/g and 0.25 log CFU/g, respectively.

Significance: The slight decrease in *B. cereus* populations observed over the 24-hour cooling period combined with no significant difference (*P*>0.05) in *B. cereus* population observed for the cover (two layers, one layer, uncovered) variable indicate that all cooling techniques were effective at controlling *B. cereus* population outgrowth in prepared rice.

P2-78 A Survey of the Microbiome Sampled from Surfaces of Supermarket Shopping Carts and Grocery Baskets

M. ALEXANDRA CALLE, Brayan Montoya, Andrea English and Mindy Brashears
Texas Tech University, Lubbock, TX

Introduction: Supermarkets microbiological assessments are typically performed on food, and little information is available about carts and baskets used for grocery shopping. Studies on shopping carts contamination express concerns mostly associated with infants' exposure to pathogens; however, there is the potential of food contamination due to carts and baskets.

Purpose: To identify the microbial contamination present in supermarkets shopping carts and baskets.

Methods: A total of 97 samples were collected from 21 supermarkets at eight different towns located in West Texas. Shopping cart handles, shopping cart grills, and grocery baskets were sampled using 25 ml BPW swabs. Microbial analyses were performed to enumerate aerobic plate count (APC), yeast and molds (YM), *Enterobacteriaceae* (EB), coliforms (CF), and *Escherichia coli* (EC). The prevalence of *Listeria* spp., *Salmonella* spp., *Staphylococcus aureus*, *Enterobacteriaceae*, and *E. coli* O157 was determined. Petrifilm, BAX system, and conventional selective agar media were used for the analyses.

Results: APC, EB, CF, and EC were at higher levels on handles (2.45, 4.43, 6.43, and 1.44 log CFU/cm^2, respectively), followed by baskets (2.03, 3.67, 5.55, and 0.7 log CFU/cm^2, respectively). YM was highest on baskets (3.01 log CFU/cm^2). Cart grills had less than 10 CFU/cm^2 of APC, YM, CF and EC, but 2.38 log CFU/cm^2 of EB. The overall prevalence for the three type of samples was found to be: *Salmonella* 7.2%, *E. coli* O157 6.2%, *S. aureus* 61.8%, *Listeria* spp. 76.3%, (8.5% confirmed *L. monocytogenes*), and *Enterobacteriaceae* 51.5%.

Significance: Bacterial contamination of shopping carts and grocery baskets should be treated as a major public health threat. Fruits and vegetables are often placed on carts and baskets without protection; similarly, meat and other foodstuffs are wrapped in bags not properly sealed during grocery shopping. The presence of foodborne pathogens on such surfaces pose a risk of food contamination and a potential cause of illness.

P2-79 Evaluating the Self-perception of Skills and Roles of Third-party Food Safety Auditors in Grocery Stores

NATALIE SEYMOUR, Kimberly Allen, Eric Laber and Benjamin Chapman
North Carolina State University, Raleigh, NC

Introduction: It is common for food companies to institute standard operating procedures based on the best available science that exceed regulatory requirements for food safety. Third-party auditors often evaluate these risk reduction procedures. A proposed strategy to increase adherence to operating procedures is to enable and support third-party auditors to deliver behavior change interventions during audits. However, no data exists on auditors' self-perception of their roles and abilities.

Purpose: The goal of this study was to characterize third-party auditors' self-perception of job roles and skills, and attitudes relating to promoting food safety. This study also sought to identify whether auditors were currently providing interventions, methods they may use and perception of how intervention efforts are received.

Methods: A mixed-methods online survey was developed with questions targeting the attitudes towards roles of auditor, teacher, and coach; intervention strategies; and, importance of job activities. Auditors currently employed by a leading food safety auditing service provider were asked to participate via email. Data was analyzed with one-way analysis of variance and Tukey's honest significance test to determine significant differences. Tests looked for frequency as well as differences based off experience or prior answer selection.

Results: Auditors more closely identify with the roles of teacher (91%, (n=121) and coach (90%) but value activities of an auditor. Auditors report protecting public health and identifying problems as most important out of nine job activities, food safety knowledge was also a top priority. Ninety-two percent reported providing an intervention when observing non-compliant behavior; 46% pull from previous audits for interventions.

Significance: Results demonstrate auditors are confident in their abilities to explain food safety information and believe retail employees view them and their efforts positively. These attitudes lend to using these individuals to deliver real-time interventions to promote positive food safety behavior change.

P2-80 A Simulation Study to Evaluate the Microbiological Safety of School Lunches Stored in Insulated Coolers during Field Trips

NICHOLAS SEVART[1], Sara Gragg[2], Paola Paez[1], Amanda Wilder[1], Tracee Watkins[1] and Randall Phebus[1]
[1]Kansas State University, Manhattan, KS, [2]Kansas State University, Olathe, KS

Introduction: Field trips, often during warm weather seasons, present a food safety challenge for school nutrition programs, as providing a nutritious meal that has been stored and handled properly until serving may be difficult. Sack lunch meals are often stored in insulated coolers that accompany students on the field trip. The coolers are commonly stored on school buses, which are not temperature regulated, and may pose a risk to food safety.

Purpose: Understanding foodborne pathogen growth in school lunch meals that have been packaged in insulated coolers, transported on field trips, and exposed to elevated environmental temperatures prior to serving was the purpose of this research.

Methods: Turkey sandwiches, baby carrots, and sliced apples were inoculated with *Listeria monocytogenes* or *Salmonella* spp., packaged into sack lunches, and arranged in a cooler with no ice packs, or a cooler with one layer of ice packs on the bottom. Packed coolers were placed in a commercial smokehouse and subjected to a five-hour program that simulated temperature increases within a school bus on a hot day. Product samples were analyzed by direct selective plating to quantify changes in initial pathogen levels after the applied storage treatment.

Results: Thermocouple data from both coolers indicate that food products were subjected to temperatures conducive for foodborne pathogen growth (top layers reaching up to 32°C after 5 h). However, no pathogen growth was observed during the five-hour storage simulation in any food product in either cooler packing scenario (P>0.05).

Significance: Although sandwiches, carrots, and apple slices were subjected to temperature abuse in both cooler packing scenarios, pathogen populations did not increase during the five-hour simulation. Therefore, storage time as a public health control is effective for preventing foodborne pathogen growth on these products.

P2-81 Food Allergy Information Sharing and Communication Strategies in Full-service Restaurants in the United States

HAN WEN[1] and Junehee Kwon[2]
[1]University of North Texas, Denton, TX, [2]Kansas State University, Manhattan, KS

Introduction: Foods prepared in restaurants were responsible for a significant number of fatal food allergy reactions in the United States. Miscommunication between and among restaurant staff and customers was perceived as one of the major causes of allergic reactions. However, customers with food allergies did not willingly share their special needs with staff for various reasons. Establishing proper communication with these customers may help prevent food allergy reactions.

Purpose: This study was conducted to explore how restaurant staff shares food allergy information and communicates risks with customers with food allergies.

Methods: An online survey instrument was developed based on interviews and literature review, reviewed by an expert panel, and pilot-tested before data collection. An online survey company distributed the survey link to a restaurant employee panel to reach full-time restaurant service staff. Descriptive statistics and content analysis of open-ended responses were conducted.

Results: A total of 316 usable responses were collected. Only 27 (8.5%) servers reported that their restaurants had separate menus for customers with food allergies, yet 204 (64.6%) servers stated that they never or rarely asked if customers had any food allergy. Informing customers when the food preparer is unable to provide allergen-free meals (5.76±1.39 of 7 point scale), including a statement on the menu advising customers to notify the server about their food allergies (5.58±1.44), and having a written protocol with procedures for serving customers with food allergies (5.52±1.42) were perceived as the three most effective communication strategies.

Significance: Despite the risk of allergic reactions due to cross-contacts when special needs are not communicated, restaurateurs depended on written, one-way communication rather than proactively initiating communications. It is suggested that restaurateurs train employees to establish two-way communication, including reassuring customers about their allergen-free orders and to develop a written protocol detailing the communication procedures with customers with food allergies.

P2-82 Measuring and Modeling the Influence of Temperature and Relative Humidity on the Survival of *Enterobacter aerogenes*

MATTHEW IGO[1] and Donald W. Schaffner[2]

[1]Rutgers University, Medford, NJ, [2]Rutgers University, Department of Food Science, New Brunswick, NJ

Introduction: The survival of microorganisms on food contact surfaces is an important part of understanding cross-contamination. Temperature, relative humidity, and surface type all appear to play a role. Primary and secondary models for describing microbial behavior under these circumstances are limited.

Purpose: The purpose of this study was to measure and develop primary models for the effects of temperature and relative humidity on the survival of the nonpathogenic surrogate *Enterobacter aerogenes* (B199A).

Methods: Stainless steel tiles were inoculated with approximately six log CFU *E. aerogenes* per tile and dried for two hours at room temperature. Tiles were placed in desiccators containing saturated salt solutions at ~15, 50, and 100% relative humidity at 7 and 21°C. Tiles were sampled at appropriate time intervals ranging from 8 h to 21 days. Samples were plated in duplicate and experiments were repeated in triplicate. Survival modeling was conducted using DMfit software.

Results: *Enterobacter aerogenes* survival generally showed a decline followed by a plateau and could be modeled using Biphasic or Baranyi and Roberts models with no lag. The R^2 values for the primary models ranged from 0.60 to 0.89 indicating relatively good fit. Final concentration of *E. aerogenes* at 7°C were 4.5, 4.9, and 5.0 log CFU/surface after 21 days at 15, 50, and 100% RH, respectively. *Enterobacter aerogenes,* generally, did not survive as well at 21°C with final concentrations of 1.3 and 2.7 CFU/tile at 15 and 50% RH, respectively; but showed either growth or injury recovery at 100% RH, with a final concentration of 6.5 log CFU/tile.

Significance: These results show the potentially complex interactions between temperature and relative humidity on survival of microorganisms. More research is needed to develop secondary mathematical models for survival of microbes on surfaces at different temperatures and environmental RH values.

P2-83 Trends in Food Safety in Food Trucks across Dubai

SHUGUFTA MOHAMMAD ZUBAIR[1], Muhammad Khalid Saeed[1], Ahmed Rashid Al Ani[1] and Shelendra Singh[2]

[1]Dubai Municipality, Dubai, United Arab Emirates, [2]Starwood Hotels & Resorts (Sheraton Hotels), Dubai, United Arab Emirates

Introduction: Due to Dubai's constant efforts, it is one of the growing tourist destination that attracts visitors from the western world. Recently the region of Dubai has seen a growing number of food trucks. The food truck park is a new venture in the list of tourist places in Dubai.

Purpose: The purpose of this study was to evaluate the food safety standards and trends in Dubai food truck establishments and determine the top five food safety violations associated with these trucks.

Methods: Various food trucks in different regions of Dubai were analyzed. Some were permanent establishments and others were functional during events. Parameters were set based on the new inspection system of Dubai municipality. As calculated, A=95+, B=80+, C=70+, and D=60. Scores were based on the number of critical, major, and general violations. A survey using the Dubai municipality food code was prepared and inspections were carried out in temporary and permanently established food trucks across Dubai. The survey results (n=50) were analyzed using ANOVA and plotted based on the samples collected.

Results: The results indicate that there is a difference (P-value) in the number of violations and compliance to food safety between the trucks functional during events and the permanently established trucks. The event based trucks are less compliant to food safety standards than the permeant food trucks and the mean of the violations are 0.56 and 0.68, respectively, with the P<0.05 significance. Also the variances of grades were consistently found to be B grades in the permanent trucks, whereas temporary event-based trucks received scores that varied from B to D.

Significance: These data helps the regulatory authority in analyzing the food safety aspects and devising comprehensive solutions and guidelines that can be utilized by the food truck establishments.

P2-84 Aerobic Plate Counts and Contact Surface Category Correlate with the Presence of *Listeria monocytogenes* in Retail Grocery Produce Environments

DEKLIN VEENHUIZEN[1], John Burnett[1], Tongyu Wu[1], Susan Hammons[1], Manpreet Singh[2] and Haley Oliver[1]

[1]Purdue University, West Lafayette, IN, [2]University of Georgia, Athens, GA

✦ ***Undergraduate Student Award Competitor***

Introduction: Listeriosis from fresh produce is an emerging safety risk. Our group found a positive correlation between standard aerobic plate count (APC) and the probability of detecting *Listeria monocytogenes* in retail delis. This relationship is unexplored in retail produce environments.

Purpose: The purpose of this study was to determine if APC can be predictive of the probability of detecting *L. monocytogenes* on food and nonfood contact surfaces.

Methods: Twenty environmental swabs were collected monthly for three months from 20 retail produce departments across seven states (n=1,041). Sample sites included nine nonfood contact (NFCS) and 11 food contact surfaces (FCS). Prior to enrichment, an aliquot from each environmental sample was serially diluted and plated on Petrifilm™ aerobic count plates. Samples were subsequently tested for *L. monocytogenes* using the ROKA Atlas®LmG2 assay with confirmation of positives through secondary enrichment in Fraser broth followed by plating on RAIPID'L.mono plates.

Results: The highest APC were from floors, drains, squeegees, organic shelving, and standing water. A total of 7.7% (80 of 1,041) of samples were confirmed positive for *L. monocytogenes;* specifically, *L. monocytogenes* was found on 1.8% (10 of 571) of FCS and 14.9% (70 of 470) of NFCS. Using logistic regression, the odds of detecting *L. monocytogenes* increased 1.8-fold for every one-log increase in APC (P<0.0001). Surface type had an additional, separate effect, in that there was an 8.1-fold increase in the odds of detecting *L. monocytogenes* on NFCS versus FCS (P<0.0001).

Significance: This study supports that APC may be a cost-effective environmental monitoring tool to identify sanitation challenges that could potentially result in *L. monocytogenes* harborage. Using APC as a sanitation monitoring tool may have an additional benefit of indicating an opportunity for shelf life extension and food quality improvement.

P2-85 Evaluation of the Survival of *Salmonella* spp. and *Escherichia coli* O157:H7 in Unpasteurized Apple Juice from Juice Bars

DON MACK[1], Shakaree Hale[1], Autumn Woods[1], Marciauna Daniel[2], Lamin Kassama[2], Aubrey Mendonca[3] and Armitra Jackson-Davis[4]
[1]Alabama A&M University, Huntsville, AL, [2]Alabama A&M University, Normal, AL, [3]Iowa State University, Ames, IA, [4]Alabama A&M University, Madison, AL

Introduction: Juice bars continue to gain popularity. Because unpasteurized juice served in juice bars is not thermally processed, these products may contain pathogens. Although the safety of unpasteurized juice has been studied, research has not focused on the microbiological safety of juice produced in juice bar conditions.

Purpose: The objective of this study was to evaluate the survival of *Salmonella* spp. and *Escherichia coli* O157:H7 in unpasteurized juice at two different juice bars.

Methods: Unpasteurized apple juice (Brands A and B) was purchased and inoculated with *Salmonella* spp. and *E. coli* O157:H7 (10^8 CFU/ml) and stored at 4°C and 23°C. Survival was evaluated over 14 days. Samples were plated on XLD and SMAC and incubated (37°C, 24 hr). Brands C and D served as pasteurized controls.

Results: As it relates to *Salmonella* spp., the Brands significantly affected (P<0.01) growth. Brand B displayed the highest pH (4.21) while having the lowest log reduction (3.13 log CFU/ml). A reduction of 4.06 log CFU/ml was observed in Brand D, which is significantly different (P<0.01) from Brands A (3.32 CFU/ml) and B (3.14 log CFU/ml). The microbial load of Brand C was 3.69 log CFU/ml. Brands held at 4°C were significantly different in population compared to brands held at 23°C. As it relates to *E. coli* O157:H7, the highest inhibition of *E. coli* O157:H7 (P<0.05) was observed in Brands C and D compared to Brands A and B. The results showed a significant (P<0.05) reduction of *E. coli* O157:H7 populations by 3.62 log in Brand D, 3.31 log CFU/ml in Brand C, 0.25 log CFU/ml in Brand B, and 1.52 log CFU/ml in Brand A when stored at 4°C. Greater inhibition was observed at 4°C than at 23°C.

Significance: Unpasteurized apple juice in juice bars has more potential for survival of *E. coli* O157:H7 and *Salmonella* spp.

P2-86 Understanding Consumers' Perceptions of Ethnic Restaurants: An Application of Importance-performance Analysis

PEI LIU and Eliza Tse
University of Missouri, Columbia, MO

Introduction: Importance-performance analysis (IPA) has been used to examine consumers' self-perceived importance and performance of the attributes, which is beneficial to prioritize improvements to the quality of food and service in the restaurant industry.

Purpose: This study identified the importance and performance of food safety related attributes among ethnic full-service restaurants' selection factors in the United States.

Methods: An online questionnaire was developed based on literature review, including respondents' demographics, importance of 32 restaurant attributes, and respondents' perceived performance of these attributes on an ethnic full-service restaurant they recently visited. A market research company was used for data collection. The mean importance ratings and mean performance values for each attribute were calculated and plotted into these quadrants.

Results: Of 208 respondents, 56.7% (n=118) were male and 77.4% (n=161) were Caucasian. Approximately 30.8% (n=64) visited a full-service restaurant two to three times per month. The three most important attributes of ethnic full-service restaurants were food safety (6.17±1.45), food taste (6.11±1.44), and cleanliness of the dining room (6.03±1.38). The participants perceived that the ethnic full-service restaurants they visited performed well in accurate guest check (5.95±1.01), serve food as ordered (5.91±1.12), and food taste (5.88±1.10). Performance of food safety (5.68±1.12) and cleanliness of the dining room (5.53±1.21) were rated as the eighth and sixteenth of 32 attributes, respectively. Three attributes (i.e. cleanliness of the restrooms and fair price) were captured in the concentration quadrant. Thirteen attributes (i.e. food taste and serve food as ordered) were categorized as "Keep up the good work".

Significance: This study illustrated using IPA as a managerial tool to identify areas, including food safety, with which more effort should be taken in ethnic full-service restaurants.

P2-87 Food Safety at Farmers' Markets: A Knowledge Synthesis of Published Research

IAN YOUNG[1], Abhinand Thaivalappil[1], Danielle Reimer[1] and Judy Greig[2]
[1]Ryerson University, Toronto, ON, Canada, [2]Public Health Agency of Canada, Guelph, ON, Canada

Introduction: Farmers' markets are increasingly popular venues in North America for the sale of fresh produce and other foods. However, the nature of their operation can present possible food safety issues, challenges, and risks to consumers.

Purpose: A knowledge synthesis was conducted, using systematic and transparent methods, to identify, characterize, and summarize published research on the microbial food safety issues and implications associated with farmers' markets.

Methods: A scoping review was conducted consisting of a comprehensive search strategy (implemented in eight bibliographic databases and verified), relevance screening, article characterization, and data charting. Two subsets of data were prioritized for more detailed systematic review (data extraction and risk-of-bias assessment) and meta-analysis: 1) studies comparing the microbial safety of foods from farmers' markets vs. other sources; and 2) studies evaluating the use of food safety practices at farmers' markets. Two independent reviewers conducted each step.

Results: The majority of the 82 relevant studies identified used a cross-sectional design (81%) and were conducted in the United States (78%). Most studies (38%; n=32) investigated stakeholder attitudes toward food safety at farmers' markets. Studies evaluating the use of food safety practices at farmers' markets (n=13) identified some gaps; for example, the average prevalence of vendor handwashing was 4% (95% CI: 0 to 11%; I^2=27%; n=5 studies). Twelve foodborne outbreaks and case reports were identified, resulting in a total of 411 illnesses, 38 hospitalizations, and two deaths from 1994 to 2016. Only five intervention studies were identified.

Significance: This review highlighted the current state of evidence on food safety at farmers' markets. Key research gaps included studies evaluating food safety practices, barriers, and needs among market vendors and managers, as well as interventions to improve food safety at farmers' markets.

Poster

P2-88 *Listeria monocytogenes* is Prevalent in Retail Grocery Produce Environments, but *Salmonella enterica* is Rare

JOHN BURNETT[1], Tongyu Wu[1], Susan Hammons[1], Deklin Veenhuizen[1], Manpreet Singh[2] and Haley Oliver[1]
[1]*Purdue University, West Lafayette, IN,* [2]*University of Georgia, Athens, GA*

❖ ***Developing Scientist Competitor***

Introduction: There has been a notable increase in produce-related foodborne illness, including recent outbreaks attributed to *Listeria monocytogenes* and *Salmonella enterica*. Recent studies show retail environments may be a source of contamination for deli meat by *L. monocytogenes*. The environmental prevalence of *Listeria* and *Salmonella* in retail produce remains largely uncharacterized.

Purpose: The purpose of this study was to determine the prevalence of *L. monocytogenes* and *S. enterica* in retail produce environments and to elucidate possible ecological niches.

Methods: Thirty environmental samples were collected monthly for six months, in duplicate, in 30 retail produce departments in seven states during daily operation. Samples included 17 food contact surfaces and 13 nonfood contact surfaces. Each sample was tested for *L. monocytogenes* and *S. enterica* using ROKA Atlas LmG2 and SEN assays, respectively. Positive *L. monocytogenes* enrichments were confirmed by secondary enrichment in Fraser broth and plating; positive *S. enterica* enrichments were confirmed using the standard FDA BAM protocol.

Results: A total of 4.4% (226 of 5,112) of environmental samples tested positive for *L. monocytogenes*. *Listeria monocytogenes* was present on 8.1% (178 of 2,205) nonfood contact surfaces and 1.6% (48 of 2907) of food contact surfaces tested. Seven of 30 stores showed low overall *L. monocytogenes* prevalence across all sites (<1%) and four of 30 stores had high overall prevalence (>10%). The majority of positive *L. monocytogenes* samples were found on the drains or floors, squeegees, and in standing water. *Salmonella enterica* was found during one sampling event in a single store; <0.1% (four of 5,112) of samples were positive overall.

Significance: This study suggested that retail environments may be a significant source of *L. monocytogenes,* which may result in cross-contamination to produce. However, the data indicated that *S. enterica* prevalence, in retail, is very low and likely due to transient contamination events.

P2-89 Persistence of *Escherichia coli* O157:H7 on Commonly Used Food Packaging Materials

DULEEKA KURUWITA ARACHCHIGE[1], Xiuping Jiang[1], Angela Fraser[1], Duncan Darby[1] and Julia Sharp[2]
[1]*Clemson University, Clemson, SC,* [2]*Colorado University, Fort Collins, CO*

Introduction: *Escherichia coli* O157:H7 causes many foodborne disease outbreaks in the United States. Because of its low infectious dose (<10 cells), contaminated surfaces, such as the outside of a food package, could be a source for disease transmission.

Purpose: Our aim was to determine persistence of *E. coli* O157:H7 on three food packaging materials: oriented polyethylene terephthalate (OPET), oriented polypropylene (OPP), and nylon 6.

Methods: Coupons (25 cm^2) from each material were sterilized under ultraviolet light for five minutes. Spot and spread inoculation was performed on treatment coupons with ca. seven log CFU of a three strain mixture of green fluorescent protein (GFP)-labeled *E. coli* O157:H7. All were incubated as per Technical Association of the Pulp and Paper Industry (TAPPI standards: 23°C temperature; 50% relative humidity). Surviving *E. coli* O157:H7 cells, on duplicate coupons, were recovered in saline at selected time intervals (0, 0.25, 0.5, 1, 2, 3, 5, 7, 14, and 15 days) and enumerated on tryptic soy broth + ampicillin, using the three tubes most probable number (MPN) method described in the Bacteriological Analytical Manual. Day 30 coupons were enriched in tryptic soy broth + ampicillin. Isolated colonies were confirmed using the latex agglutination method. The experiment was performed in triplicate.

Results: GFP-labeled *E. coli* O157:H7 survived on OPET, OPP, and nylon 6 for over two weeks. Survival of *E. coli* O157:H7 rapidly decreased until day two on OPET (7.38±0.33 log MPN to 4.40±0.31 log MPN), OPP (7.50±0.41 log MPN to 3.83±0.64 log MPN), and nylon 6 (7.34±0.52 log MPN to 4.12±0.37 log MPN). The survival of *E. coli*O157:H7 on all three materials was not significantly different.

Significance: *Escherichia coli* O157:H7 survived for over two weeks on OPET, OPP, and nylon 6, suggesting the outer surface of a food package could be a potential fomite.

P2-90 Leafy Greens: Risk Reduction and Industry-related Interventions

KRISTINA INFANTE[1] and Sujata A. Sirsat[2]
[1]*University of Houston, Conrad N. Hilton College of Hotel and Restaurant Management, Houston, TX,* [2]*University of Houston, Houston, TX*

✦ ***Undergraduate Student Award Competitor***

Introduction: Raw leafy-greens are a power packed, nutritious food; however, there have been multiple foodborne disease outbreaks associated over the past years.

Purpose: The goal of this study was to design practical methods and improve sanitation protocols to reduce the levels of pathogenic contamination on fresh produce in restaurant kitchens.

Methods: Lettuce was purchased from local grocery stores and inoculated with a pathogenic cocktail composed of *Listeria monocytogenes* (LM), *Salmonella* Typhimurium (ST) and *Escherichia coli* O157:H7 (EC). Romaine lettuce (100 g) was added to 50 ml of 10^8CFU/ml inoculum cocktail in a sterile beaker. The lettuce was dried for one hour in a biosafety cabinet, followed by the analysis of the 30 s, 2 min, and 5 min water-wash submersion controls. Intervention analyses were conducted as follows: USDA certified organic vegetable wash submersions (30 s/2 min/5 min), and 1:1 acid to oil dressing (including acetic, citric acid, and olive oil; 30 s/2 min). Methods for isolation and enumeration were in accordance with the Bacteriological Analytical Manual. Samples were aseptically stomached with 0.1% peptone buffer for 120 s, serially diluted, and plated on EMB (ST and EC enumeration) or PALCAM agar (LM) enumeration.

Results: Samples exposed to the oil and acid dressing for 30 s showed a reduction in ST and EC counts to below the detectable limits (5.6-log CFU/g reduction) and a 2.4-log CFU/g reduction in LM. Samples submerged in the USDA certified organic vegetable wash for 30 s showed a reduction in ST and EC counts by 1.4 log CFU/g and LM by 0.8 log CFU/g.

Significance: The use of practical, inexpensive protocols for leafy green sanitation could enhance food safety practices within the restaurant industry, especially while catering to immunocompromised individuals. It is, also, especially crucial to design and disseminate educational materials focused on science-based interventions to stakeholders.

P2-91 Influence of Cooling Rate on Growth of *Bacillus cereus* from Spore Inocula in Cooked Rice, Beans, Pasta, and Combination Products

VIJAY JUNEJA[1], Timothy Mohr[2] and Oscar Snyder[3]

[1]U.S. Department of Agriculture–ARS, Wyndmoor, PA, [2]Science Staff/OPHS/FSIS/USDA, Salem, OR, [3]SnyderHACCP, Shoreview, MN

Introduction: *Bacillus cereus* continues to be a pathogen of concern to the retail food service industry. Inadequate cooling practices and/or improper storage of rice, beans, pasta, and multicomponent meat and poultry products have been cited as a cause of numerous outbreaks of foodborne illness.

Purpose: This work studied the ability of *B.cereus* spores to germinate and grow, in order to determine a safe cooling rates for cooked rice, beans, pasta, rice/chicken (4:1), rice/chicken/vegetables (3:1:1), rice/beef (4:1), and rice/beef/vegetables (3:1:1).

Methods: Samples were inoculated with a cocktail of four strains of heat-shocked (80°C/10 min) *B. cereus* spores (NCTC 11143, 935A/74, Brad 1, and Mac 1) to obtain a final spore concentration of approximately two log CFU/g. Thereafter, samples were cooled through the temperature range of 54.5°C to 7.2°C in 6, 9, 12, 15, 18, and 21 h. At the end of the cooling period, samples were removed and plated on mannitol egg yolk polymyxin agar. The plates were incubated at 30°C for 24 h.

Results: While minimal growth was observed in samples from the six hour cooling period, greater than one log CFU/g growth from *B. cereus* spores was observed in all products during nine hour cooling. The only exception was beans in which, when the time to achieve 7.2°C was extended to 12 h, *B. cereus* spores germinated and grew from an inoculum of 2.2 log to 4.0 log CFU/g. A public health concern was noted when the extent of growth from spores, in all products, was up to three log CFU/g during cooling to 7.2°C in 15 h.

Significance: The study results suggested safe cooling rates for cooling cooked rice, beans, pasta, rice/chicken, rice/chicken/vegetables, rice/beef and rice/beef/vegetables to guard against *B. cereus* foodborne disease outbreaks.

P2-92 Vomit and Diarrhea Clean-up Practices at Foodservice and Retail Food Establishments

CATHERINE VIATOR[1], Morgan Chao[2], Sheryl Cates[3], Jonathan Blitstein[3], Megan Clayton[3], Cortney Leone[2], Kinsey Porter[2] and Angela Fraser[2]

[1]RTI International, Houma, LA, [2]Clemson University, Clemson, SC, [3]RTI International, Research Triangle Park, NC

Introduction: Clean-up of vomit and diarrhea at foodservice and retail food establishments is critical to prevent the transmission of viruses that cause illness, such as noroviruses.

Purpose: This study assessed the frequency of vomit and diarrhea events at foodservice and retail food establishments and determined employees' use of recommended clean-up procedures before participating in a training intervention.

Methods: A survey of foodservice and retail food employees, who were enrolled in a food safety manager training course in summer/fall 2016 (*n*=679), was conducted. Respondents were from states that have adopted the 2009 or 2013 Food Code.

Results: Thirteen percent of surveyed establishments experienced a vomit event in the past three months, and six percent experienced a diarrhea event in the past three months. Approximately half of the establishments had written clean-up procedures (49%), and of those, half (52%) train their employees on the clean-up procedures. The following steps are specified in the written procedures: blocking off contaminated area (76%); covering the event with paper towels/absorbent powder (74%); washing (76%) and disinfecting (81%) contaminated surfaces; disposing of personal protective equipment (PPE) (83%); and machine washing all soiled linens, towels, and clothing (72%). Fewer establishments (36%) have a clean-up kit, although an additional 34% plan to purchase or assemble one within the next year. Establishments included the following PPE and supplies in their procedures: disinfectant (84%), paper towels (80%), disposable gloves (72%), disposable hair covers (48%), absorbent powder (36%), disposable gown with sleeves (31%), and shoe covers (25%).

Significance: Although vomit and diarrhea events are not common, only half of the establishments surveyed are prepared with written clean-up procedures, and only one-third have a designated clean-up kit. We will analyze the results of the forthcoming follow-up survey to determine the effectiveness of a new training module on vomit and diarrhea clean-up.

P2-93 Nears and Nors Merge: A Preliminary Analysis

KRISTI-WARREN SCOTT

Centers for Disease Control and Prevention, Atlanta, GA

Introduction: The National Environmental Assessment Reporting System (NEARS) managed by the National Center for Environmental Health's Safe Food Team, and the National Outbreak Reporting System (NORS) managed by the National Center for Emerging Zoonotic and Infectious Diseases are CDC's two primary foodborne illness reporting systems. Each system is designed to collect different information; while NORS collects clinical and epidemiological data on foodborne illness outbreaks, NEARS collects environmental data related to foodborne illness outbreaks. Previous food safety studies have used each of these datasets, yet no known studies exist that utilize both datasets.

Purpose: This study is based on the merged datasets and aims to describe restaurant-related norovirus outbreaks using NEARS data (2009 to 2014) and NORS outbreak data (2009 to 2014). Descriptive analyses and inferential statistics help to provide more information about the foodborne illness outbreaks during 2009 to 2014.

Methods: Descriptive statistics include mean, median, and distribution statistics of characteristics of outbreak restaurants. Cross-tabulation statistics show mean norovirus cases by outbreak restaurant characteristics and food safety policies. Lastly, inferential statistics examine statistically significant relationships between norovirus cases and outbreak restaurant food safety policies. The data were merged, cleaned, and analyzed using Stata SE 13.

Results: Preliminary results indicate that some restaurant policies are statistically significantly associated with norovirus outbreak size. Particularly, restaurants with ill food worker communication policies had fewer average confirmed primary norovirus cases than restaurants without these policies.

Significance: The decision to merge the NEARS and NORS datasets is primarily motivated by the aim to expand food safety analysis possibilities. Currently, no known dataset includes both food safety environmental and epidemiology indicators, so a merge allows CDC researchers to conduct analytic studies to examine the relationship between food safety environmental and etiologic variables of interest and thus identify effective interventions and reduce outbreaks.

P2-94 Removal of Human Pathogens from Surfaces Using a Novel Microfiber Towel

KEVIN MO, Victor Pool, Holly Paden and Sanja Ilic
Ohio State University, Columbus, OH

✦ ***Undergraduate Student Award Competitor***

Introduction: Food safety continues to be of the highest importance to public health. Even though consumption in restaurants continues to grow in the United States, with one half of total food expenses in 2013 spent on food away from home, foodborne illnesses linked to restaurant contamination continue to be high. In foodservice, surfaces can easily become contaminated with human pathogens from raw foods or food handlers. Effective cleaning of food contact and dining surfaces is essential to prevent contamination. Novel microfiber single use cleaning towels are available in the marketplace for use in foodservice. However, their efficacy in removal of human pathogens is not known.

Purpose: The objective of this study was to determine the effectiveness of a novel microfiber towel in removing *Escherichia coli* O157:H7 and *Salmonella* spp. from foodservice surfaces.

Methods: A total of 80 trials were completed to test removal rates of *E. coli* O157:H7 and *Salmonella* Typhimurium from stainless steel and acrylic surfaces with one-use dry microfiber towel. After spot inoculation of surfaces, the removal rates from wet and dried contamination surfaces were measured. Each test was repeated 10 times. Pathogen concentrations were calculated and data was analyzed in SPSS.

Results: The microfiber towel removed pathogens from stainless steel (54±15%) and acrylic (52±17%, $P>0.05$) with similar efficacy. The removal rate from dry surfaces was lower (48±18%) than when the surface was wet (58±10%, $P<0.05$). Overall, the log CFU reduction was greater per wipe for *Salmonella* Typhimurium (4.6±0.9 log CFU) than it was for *E. coli* O157(4.0±1.2 log CFU, $P<0.05$).

Significance: Novel microfiber towels may be an effective intervention to prevent cross-contamination of human pathogens in foodservice. The findings of this study can be used by the industry to assess the efficacy of sanitation practices and develop effective management procedures for foodservice.

P2-95 pH of State Fair Entries in North Carolina

KATRINA LEVINE[1], Benjamin Chapman[1] and Donald W. Schaffner[2]
[1]*North Carolina State University, Raleigh, NC,* [2]*Rutgers University, Department of Food Science, New Brunswick, NJ*

Introduction: In North Carolina, judges taste-test home preserved foods submitted for competition at the State Fair. Beginning in 2014, all entries were required to include a copy of the recipe to verify it was made from an evidence-based recipe from educational or governmental sources. Little is known about the variability of pH in home preserved products submitted to the NC State Fair.

Purpose: The purpose of this exploratory research is to show the variation in types of foods and the pHs of those foods among home preserved foods submitted to the NC State Fair.

Methods: Information on preserved product categories and pH was collected from high-risk NC State Fair entries in 2015 and 2016. Information on recipe sources was collected in 2016 only.

Results: In 2016, the most frequently used evidence-based recipe source was the Ball Blue Book (34%, $n=27$). However, nearly half (45%; $n=36$) of recipe sources were unknown. Five entries (6%) provided non-evidence based sources for either the recipe or processing method. The pH ranged from 2.73-5.21 in 2015 and 2.70-4.43 in 2016. Butters had the highest mean pH in 2015 (4.54; $n=3$); this average decreased to 3.52 in 2016 ($n=9$). Preserves had the highest mean pH in 2016 (3.82; $n=6$), which was still below the safety threshold of 4.6. In 2015, two entries were above the safe pH threshold. Both of these entries were in the butter category and had pHs of 4.88 and 5.21.

Significance: Home-preserved butters are high-risk products and can have a pH above the safe threshold. These results can inform judging regulations for competitions where home-preserved products are taste-tested.

P2-96 Evaluation of a Training Program for Volunteer Food Handlers

KATRINA LEVINE, Benjamin Chapman and Dara Bloom
North Carolina State University, Raleigh, NC

Introduction: Food safety training for volunteer food handlers is lacking. The Extension Master Food Volunteer (EMFV) Program was created in North Carolina to provide training on food-related topics, including food safety, to volunteer food handlers who assist family and consumer sciences (FCS) extension agents. Other food-related topics include cooking skills, nutrition, and food systems. A pilot of the EMFV Program was implemented in five North Carolina counties in 2016.

Purpose: The purpose of this study was to evaluate this train-the-trainer volunteer food handlers training program for efficacy as a food safety knowledge change and skill building intervention.

Methods: A posttraining evaluation was given to volunteers and agents. Structured questions measured knowledge change, skills, and aspirations related to preparation to deliver materials to others. T-tests were performed on knowledge change scores. Exams assessed posttraining volunteer knowledge related to indicators of doneness, handwashing, home food preservation, product dating, and safe temperatures.

Results: Eight FCS Agents and 23 volunteers completed the food safety training evaluation. Both agents and volunteers improved their knowledge most regarding safe temperatures related to storing, transporting, and preparing food (57% and 91% improved, respectively). Mean knowledge ratings improved for both agents and volunteers (agents: 0.04 to 1.00 Likert scale points, SD 1.51 to 1.76; volunteers: 1.10 to 1.60 Likert scale points, SD 0.57 to 0.95). Results for both agents and volunteers were not significant due to small sample size. The majority of volunteers were able to correctly answer questions regarding indicators of doneness (95%; $n=20$), handwashing (100%; $n=21$), home food preservation (100%; $n=21$), and product dating (53%; $n=10$). Most participants incorrectly answered questions regarding time/temperature considerations (78.6%; $n=11$).

Significance: The EMFV Program has some efficacy as a knowledge change program for training volunteer food handlers on safe food handling best practices, however additional evaluations are needed to evaluate sustained knowledge and self-reported skills.

P2-97 Assessing Food Safety Knowledge, Attitude, and Practices among Florida Master Gardener Volunteers

Jing Guo, Beth Gankofskie, Candice Stefanou, Wendy Wilber, **AMY SIMONNE** and Anne Mathews
University of Florida, Gainesville, FL

Introduction: Master Gardener (MG) is one of the most well-known volunteer programs in North America. Most MG volunteer training curricula cover a wide range of subjects related to horticulture. In return, the volunteers provide services to counties by assisting with horticulture oriented projects such as community and school gardens. Food safety is not a major focus for Florida MG volunteers, even though they often prepare food for fundraising activities. Without sufficient food safety training, this could be a potential risk.

Purpose: We proposed to assess the food safety knowledge, attitude, and practices among the active Florida MG volunteers.

Methods: A 40-item online survey was developed, validated, and distributed to the currently active volunteers in October 2016, following the current recommended research methodology. One thousand twelve completed responses were analyzed.

Results: Eighty percent of the respondents were female, age 60 or older with college or post-graduate degrees. Overall, the respondents demonstrated good knowledge about time-temperature control and some common risk factors for foodborne illness, but they were not well aware of some key minimum safe internal temperatures for specific foods. Among key behaviors, the respondents reported washing hands before cooking (68%), washing produce before consumption (76%), and using recommended thawing practices (76%). However, the respondents (80%) reportedly used a thermometer less frequently than recommended to monitor the internal temperature of meat products during cooking. Among other issues, >50% of respondents, especially the female MGs, perceived that pesticide and antibiotic residues are serious food safety problems in the United States.

Significance: This study revealed food safety knowledge gaps among Florida MG volunteers, and the necessity for food safety education along with MGs' horticulture training.

P2-98 Comprehensive Traceability and Food Recall System Workshops in Caribbean Countries

Tejas Bhatt[1], **JAHEON KOO**[1], Robert Gravani[2] and Jennie Stitzinger[1]
[1]Institute of Food Technologists, Washington, DC, [2]Cornell University, Ithaca, NY

Introduction: A robust traceability system allows an organization to trace a food product from primary production to consumption when that food is affected by a food safety issue, minimizing potential public health risks. Efforts are being made to strengthen the capacity of the Caribbean Forum (CARIFORUM) countries to proactively address food safety issues by improving regional traceability programs.

Purpose: A series of training workshops, conducted by Institute of Food Technologists' (IFT) Global Food Traceability Center (GFTC), funded by the European Union (EU) and Inter-American Institute for Cooperation on Agriculture (IICA), helped public and private sector professionals from selected CARIFORUM countries evaluate and enhance current traceability programs and strengthen awareness about the critical importance of traceability within the CARIFORUM Region.

Methods: IFT GFTC conducted two-day interactive traceability workshops in nine Caribbean countries from July through November, 2016, that included a series of lectures, dynamic tools, group exercises, and discussions. The participants received a preworkshop assessment to measure their baseline level of awareness and understanding of traceability. One week after the workshop, participants were sent a postworkshop electronic survey to measure their feedback on their level of satisfaction with the course, as well as course impact.

Results: More than 230 participants obtained technical assistance in understanding traceability basics, regulations, food product recalls, enhancing their traceability systems, and the development of a traceability action plan. Information about how a robust traceability system improves operational efficiencies, competitive advantages, and supply chain management were also discussed. Postworkshop surveys strongly indicated that sessions were very informative, including dialogue between the public and private sector representatives and provided an opportunity for networking among agro-processors participants.

Significance: The workshops helped participants evaluate their traceability programs and enhance traceability action plans to strengthen their entire food protection system and to achieve desired business outcomes.

P2-99 Lessons from the Field: Evaluation of a Vomit/Diarrhea Clean-up Intervention Targeting Foodservice and Retail Food Workers

Morgan Chao[1], Cortney Leone[1], **KINSEY PORTER**[1], Catherine Viator[2], Sheryl Cates[3], Jonathan Blitstein[3], Megan Clayton[3] and Angela Fraser[1]
[1]Clemson University, Clemson, SC, [2]RTI International, Houma, LA, [3]RTI International, Research Triangle Park, NC

Introduction: Rigorous evaluations inform the evidence base for food safety education. Unfortunately, limited information is available in the literature to guide food safety educators in how to successfully conduct rigorous evaluations in real world retail/foodservice settings.

Purpose: To identify the challenges of evaluating a vomit/diarrhea clean-up intervention targeting retail/foodservice food workers.

Methods: Best practices to plan the evaluation of a multistate vomit/diarrhea clean-up intervention were followed, starting with reviewing the literature, framing the evaluation in a theory of behavior change, developing a logic model, and conducting a power analysis for sample size estimation. The evaluation was then conducted using a quasi-experimental research design. Educators (n=34) from 20 states were enrolled then assigned to a treatment (delivered intervention as part of food safety training) or control group (delivered standard training). Pre- and postintervention surveys were administered to persons enrolled in training courses to collect data about knowledge, attitudes, and practices.

Results: Recruiting and retaining study educators was challenging. Challenges included time constraints (educators with eight-hour trainings expressed concern about including additional materials, cancelled trainings due to low enrollment, and survey administration issues (online survey could only be sent to those who had provided email addresses at sign-up and paper survey could only be administered to those who arrived early to training). It was important to engage educators in the study and frequently communicate with them to address concerns. Recruiting training subjects was also challenging. Initially, we intended to include only managers at commercial/retail foodservice establishments, however, because the eligibility rate was lower than anticipated, we relaxed our screening criteria to include institutional foodservice managers.

Significance: Our experiences can help food safety educators anticipate and avoid specific challenges when evaluating interventions targeting retail/foodservice workers.

P2-100 Health Professionals' Motivators and Barriers to Food Safety Education

YAOHUA FENG[1], Christine Bruhn[1], Shelley Feist[2] and Mary Choate[2]
[1]*University of California-Davis, Davis, CA,* [2]*Partnership for Food Safety Education, Arlington, VA*

Introduction: Consumers trust health professionals (nurses or dietitians) for accurate food safety information; however, few health professionals provide food safety education to high-risk consumers and less than ten percent use structured classes.

Purpose: The objective of the study is to investigate motivators and barriers to food safety education for health professionals and to explore preferred delivery formats and topics.

Methods: In Phase 1, structured focus groups (*n*=27) were conducted after training sessions with health professionals (mainly registered dietitians and registered nurses), from hospitals and public health agencies in Northern California. Each session was 1.5 to 2 hours. The focus group included questions about the barriers and motivators for health professionals to deliver food safety education to their clientele. Phase 2 was a web-based surveys conducted among health professionals (*n*=188) across the United States. Participants were recruited from local hospitals and the Fight BAC health professional mailing list.

Results: In Phase 1, participants agreed there was a need to enhance food safety education for the high-risk patients. They wanted to include safe handling within existing food and nutrition educational programs. The three major barriers identified were lack of management approval, patients not interested, and not enough time and resources. In Phase 2, quantitative data showed a different pattern, with 40% identifying patients not interested, 37% indicating not enough time and 27% responding not enough materials. Only 4% selected lack of management approval. The most preferred information delivery format was brochures or pamphlets with a verbal explanation (48%). The most needed educational topic suggestd was how to choose lower risk foods (31%). Most (82%) thought it was their role to provide food safety education to high-risk populations.

Significance: Health professionals are motivated to deliver food safety information to their clientele. However, they also need management level support by providing dedicated food safety time and validated educational materials that address their needs.

P2-101 Evaluation of Positive Deviance Food Safety Curriculum Among High School Students: A Pilot Study

YAOHUA FENG and Christine Bruhn
University of California-Davis, Davis, CA

Introduction: Most food safety education is delivered through lecture and reading and may not include motivators or take into account barriers faced by high school students. The positive deviance food safety curriculum, allows students to discuss their food handling behaviors under the direction of a trained moderator and to decide whether or not to try recommended practices modeled by peers like themselves.

Purpose: This report evaluated the effectiveness of the positive deviance curriculum.

Methods: Students from a high school microbiology class in Southern California (*n*=121) completed the positive deviance curriculum, including "Cook", "Chill", "Clean", "Separate", and "Choose". Evaluation of the learning experience included pre- and post surveys, take-home tasks, in-class activities, and an observation cooking lab. The mixed measurements assessed students' and their family and friends' food handling knowledge, attitudes, and practices.

Results: The evaluation documented a significant knowledge and attitude change towards safe food handling. After the classes, only 18% thought that color change was the safest way to know meat was cooked thoroughly, compared with 70% before. Their correct response that "washing raw meat under running water spreads bacteria" increased from 72% to 100%. The "Clean" take-home task directed students to record their family and friends' hand washing practices. Only 13% of the 375 observations were over 20 seconds. The 'Chill' take-home task required students to assess their home refrigerator temperature. The reported temperature ranged from 13°F to 77°F, and 40% were above 41°F. When preparing meat in the class lab, most students were observed using a food thermometer to check internal temperature; however, cross-contamination behaviors were found in half of the students.

Significance: The positive deviance curriculum effectively changed high school students' food safety knowledge and behavior. More efforts should be taken to help increase their subjective norms by providing information to parents and promoting a food safety culture.

P2-102 Evaluation of the FightBAC Food Safety Campaign: The Story of Your Dinner

YAOHUA FENG[1], Christine Bruhn[1], Shelley Feist[2] and Mary Choate[2]
[1]*University of California-Davis, Davis, CA,* [2]*Partnership for Food Safety Education, Arlington, VA*

Introduction: Foodborne illness is an important public health issue in the United States. Fight BAC launched a holiday food safety campaign: "The Story of Your Dinner" from November 2016 to February 2017 in the United States. The campaign contained a video, kid placemats, holiday recipes with food safety instructions, and turkey hand competition on social media and was developed to increase the awareness of and compliance with safe food handling practices among consumers.

Purpose: This study evaluated the effectiveness of the campaign center-piece video delivering food safety information. Safe food handling knowledge and reported behavior were assessed in people with diabetes and pregnant women.

Methods: A total of 161 participants (79% female, 45% between 18 to 24 years of age) completed the study. One hundred eleven participants were recruited from Virginia and 50 from West Virginia. Pre- and post-surveys recorded demographic information and evaluated perceived risk, food safety knowledge, and food hygiene practices before and after the campaign.

Results: Participants reported a significant increase of perceived behavior control and knowledge towards food thermometer use on both large and small cut meat preparation ($P<0.05$). After the campaign, participants' correct response rate of not washing raw meat under running water increased from 17% to 74%, maximum time food should be out of the refrigerator increased from 70% to 94%, and recommended temperature range for refrigerators increased from 56% to 86%. The top three practices that participants reported learning were, hand washing, do not wash raw meat under running water, and food thermometer use. Many reported that after viewing the video they started to use food thermometers and monitor refrigerator temperatures.

Significance: "The Story of Your Dinner" campaign increased consumers' food safety knowledge and self-reported safe handling behavior. Observation behavior measurements with participants and follow-up interviews with educators are recommended for future campaigns.

P2-103 Food Safety Knowledge of Secondary School Pupils in South Wales, UK

LEANNE ELLIS and Ellen W. Evans
ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom

Introduction: The Food Standards Agency reported that 500,000 people in the UK succumb to foodborne illness, annually. To reduce the risk of foodborne illness, consumers need to be informed of domestic food safety (FS) risks and risk-reducing behaviours. Although school pupils (11–16years) may not be currently responsible for domestic food preparation, FS knowledge/behaviours developed at this age may carry into adulthood. Determination of school pupils FS knowledge gives insight into the need for FS education of future consumers. To date, UK data detailing the FS knowledge of young adults is lacking.

Purpose: This study determined school pupil's FS knowledge.

Methods: Secondary school pupils (11-16 years old) (*n*=722) attending careers events in South-Wales completed seven multiple choice FS questions.

Results: On average, school pupils correctly answered 4/7 FS questions; 14% correctly answered all questions. Knowledge scores were significantly different ($P<0.001$) between girls (Md=5, *n*=422) and boys (Md=4, *n*=270). A significant difference was, also, observed according to age ($P<0.001$). Findings established that 60% were aware a refrigerator should operate at 4°C, 63% reported that food poisoning bacteria would multiply readily between 5–63°C. The majority (77%) identified which food product was likely to contain the most bacteria and 70% correctly identified occasions that would require hand washing. Although 72% were aware that foodborne infection can result from FS malpractices at retail, industry, and domestic environments, the domestic kitchen was identified as the most likely source. Knowledge regarding freezing was determined to be lacking, with only 55% indicating awareness that a freezer should operate at -18°C and 26% believing that freezing kills bacteria. Although more than a quarter (29%) reported studying food technology/home economics at school, no significant differences in the FS knowledge of these students was determined ($P>0.05$).

Significance: Overall, 23-40% lacked sufficient FS knowledge, suggesting a need for FS education programs targeting school pupils. Furthermore there is a need to establish where/how school pupils obtain FS information.

P2-104 Assessment of *Potluck Panic*, an On-line Game for Post-secondary Food Safety Education

ADRIENNE SHEARER[1], Dallas Hoover[1], Jeanne Gleason[2], Barbara Chamberlin[2], David Abraham[2], Pamela Martinez[2], Jeffrey Klein[1], Joan Buttram[1], Sue Snider[1] and Kalmia Kniel[1]
[1]*University of Delaware, Newark, DE,* [2]*New Mexico State University, Las Cruces, NM*

Introduction: Educational games support science learning through cognitive gains and attitudinal shifts. Food science is predominantly an elective in education; experiences outside of the classroom often shape food safety knowledge and perception.

Purpose: An on-line, educational, food safety game, *Potluck Panic*, was assessed for impact on player food safety knowledge and attitudes.

Methods: Focus group participants (*n*=40) were recruited by students enrolled in an honors section of a food science course, with IRB approval. Subjects played *Potluck Panic*and answered questions before and after play. Pre-tests consisted of knowledge-based (15), perception (20), and demographic (6) questions. Post-play tests consisted of different 15 knowledge-based questions, the same 20 perception questions, and game evaluation questions.

Results: Study subjects were predominantly between the ages of 18 and 21 years (85%), U.S. citizens (93%), and female (63%). Nearly half (48%) majored in a physical science, primarily life sciences. Fewer than half (42%) were introduced to food safety in high school; 76% indicated they learned food safety at home. Almost half (46%) reported previous use of educational games. Among all subjects, knowledge-assessment items were slightly lower post-play but not statistically significant ($P>0.05$). Subjects who scored in the lowest quartile on the pre-test showed the greatest improvement. Game play increased subjects' beliefs that scientific expertise is needed for safe food production (10% increase), industry employs professionals responsible for food safety (14% increase), and regulatory agencies rank most reliable among provided sources of food safety information (7% increase). The majority of players enjoyed the game (75%), reported increased awareness of (88%) and interest in (76%) food safety, intended to seek more information (73%), and were more interested in the food science major (54%) after game play.

Significance: *Potluck Panic* effectively illustrated food safety as an important scientific discipline and improved cognition among subjects with least food safety familiarity.

P2-105 Tablet PC-based Problem-solving Activities for Enhancing Students Food Safety Self Efficacy and Motivation

HEYAO YU, Juan Madera and Sujata A. Sirsat
University of Houston, Houston, TX

Introduction: Foodborne illness significantly affect people's health. In response to this challenge, hospitality programs in different universities have created educational programs in food safety for college students. However, besides knowledge, motivation and self-efficacy are also essential for enhancing food safety practices. Multiple studies have proved that TPCs could enhance students' motivation and self-efficacy.

Purpose: The study aimed to (1) investigate the effects of TPC-based problem-solving activities on food safety efficacy and motivation; and (2) examine factors that are related to the effectiveness of a tablet PC-based food safety problem solving activity.

Methods: A within-group experimental intervention was used in the study. The TPC problem solving activity was conducted in a food safety course, in a hospitality program with 176 students. Participants received a survey on food safety motivation, self-efficacy, and technology perceptions both prior to and after the class activity. Additionally, two focus groups were conducted to provide more information about this class activity. Repeated measure MANOVA and SEM were used in the study for data analysis.

Results: Compared to baseline data food safety self-efficacy and motivation improved significantly after the class activity for 176 participants ($F(1,247) = 17.08$, $P< .001$). The SEM showed attitude to technology ($\beta= .26$, $P < .01$) and peer-to-peer interaction ($\beta= .22$, $P < .01$) was significantly related to activity reaction and reaction was significantly related to self-efficacy ($\beta= .58$, $P < .01$) and motivation $\beta= .25$, $P < .01$). Also, the results of focus groups verified the results of MANOVA and SEM.

Significance: The results showed that TPC-based problem solving activity significantly enhanced students' food safety confidence and motivation. In addition, instructors can enhance students' food safety motivation and confidence by providing activity with appropriate technology and encouraging peer communication.

P2-106 Investigation and Outreach to Increase Public Awareness of Campylobacteriosis

HANNAH BOLINGER
North Carolina State University, Raleigh, NC

Introduction: *Campylobacter* is a leading agent of foodborne disease and the resulting illness can have severe secondary sequelae, such as Guillain-Barre Syndrome. Public awareness about this pathogen is lower than for other leading foodborne pathogens, but research into specific knowledge gaps is lacking.

Purpose: The purpose was to evaluate the level of knowledge regarding *Campylobacter* among college students at the start of a college-level Food Microbiology class. Additionally, outreach and education materials were developed in an attempt to increase public awareness regarding campylobacteriosis.

Methods: A survey was administered at the beginning of the semester to students enrolled in Food Microbiology at North Carolina State University. A website, campylobacter.fbns.ncsu.edu, was created as a platform for publishing educational materials. Additionally, links to articles published on our website or elsewhere were posted through our social media campaign on Twitter (@Campypack).

Results: An estimated 34% of students beginning a class in Food Microbiology indicated that they never heard of *Campylobacter*. Many were unaware of the prevalence of campylobacteriosis or the leading food vehicles. The website has been accessed mostly be individuals living in the USA and UK with more than 2,000 page views since May 2016. On Twitter, 147 tweets have reached more than 10,000 Twitter users since June 2016.

Significance: Gaps in public knowledge regarding *Campylobacter* are still largely undefined. However, students taking Food Microbiology have all passed a general microbiology course and one-third of these students were unaware of *Campylobacter*. The general public may be even less likely to have a good understanding of campylobacteriosis, suggesting the need for education and outreach.

P2-107 Teaching through Tweeting: Lessons Learned through NoroCORE's Social Media Campaigns

ELIZABETH BRADSHAW, Rebecca Goulter, Katie Overbey, Catharine Gensel, Benjamin Chapman and Lee-Ann Jaykus
North Carolina State University, Raleigh, NC

Introduction: Over 60% of American adults receive news through social media, and 47% use the Internet as their primary source for information on science and technology. Social media has evolved as a forum for interaction between the scientific community and the public, though data on its strategic use for science communication are still lacking.

Purpose: NoroCORE took a structured approach to social media to engage and educate the public about norovirus risks.

Methods: Since August 2014, NoroCORE has had more than sixteen unique educational campaigns utilizing one or more social media platforms. In each case, the authors first identified the major messages to be conveyed, then linked them to one or more target audiences (the general public or professional groups), letting the former attributes determine the most appropriate platform. Both unidirectional (didactic) and bidirectional (interactive) approaches to information sharing were used.

Results: Collectively, these campaigns amassed over 110,000 views and led to over 4000 engagements with the public. Most campaign messages related to the public health significance of norovirus as the most common cause of foodborne illness, or prevention and control measures. Posts had the widest reach and greatest audience participation when presented within days of the release of interesting or important research. For example, a video post on a vomiting research article amassed 38,514 views, and Facebook posts on a novel human norovirus cultivation method had 30,337 views and 1355 engagements.

Significance: Benefits of this approach to outreach included more timely engagement with stakeholders and wider audience reach. The majority of the educational materials for these campaigns were produced in-house and inexpensively, and could be recreated by other organizations. Metrics and lessons learned from each campaign are presented for the benefit of other food safety and scientific groups seeking to impact their target audiences via social media.

P2-108 How to Communicate with Consumers When Flavor Preferences and Safety Conflict

CHRISTINE BRUHN and Yaohua Feng
University of California-Davis, Davis, CA

Introduction: Dimethyl dicarbonate (DMDC) can be used in juice to reduce microbiological levels and mandatory labeling is not required by the US FDA. Companies wishing to maintain transparency may want to communicate about their processing method without raising concerns about the use of chemicals.

Purpose: This study identifies consumer response to the use of DMDC in freshly squeezed orange juice and measures preferred communication approaches.

Methods: Focus group participants who were the primary food purchaser, orange juice consumers, and not employed in the food industry were recruited. Participants were asked their sources of food safety information, response to label statements, reaction to the use of chemicals to increase safety, and preference for communicating about processing methods.

Results: Three focus groups (n=25) were recruited through a professional firm, and three were recruited from a university class on research methods (n=33). Most participants were female, age ranged from 20 years to over 65 years with incomes from less than $20,000 to over $100,000 per year. Most used the internet to obtain information on health and safety and preferred the flavor of freshly squeezed juice. Some mentioned they avoided processed foods. No Added Chemicals, No Added Sugar, and Natural were the most important labeling terms while Pasteurized was ranked lowest in importance (P<0.001). Participants thought DMDC sounded scary, but after they received additional information, most were willing to try a product in which it was used. Some felt that labeling was not necessary, but several strongly believed that the consumer had the right to be informed about their food. A label indicating that the juice was treated for quality and safety combined with a web link was rated significantly higher than other statements (P<0.001).

Significance: This study modeled an approach that the food processor could follow to communicate to the consumer in a transparent manner.

P2-109 Investigating the Role of Dietitians in the Provision of Food Safety Advice for Vulnerable Patients in the UK

ELLEN W. EVANS[1], Denise Parish[2] and Elizabeth C. Redmond[1]

[1]ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom, [2]Cardiff School of Health Sciences, Cardiff Metropolitan University, Cardiff, United Kingdom

Introduction: Immunosuppression resulting from disease (e.g.diabetes, HIV/AIDS), treatment (e.g.chemotherapy, transplant/anti-rejection drugs) or antibody decline (pregnancy), creates vulnerable-patient groups that may have an increased risk of foodborne illness. To reduce risk, vulnerable patients (VP) must be adequately informed to implement risk-reducing food safety (FS) practices. Dietitians are trusted/desired providers of food-safety information; however, limited research has investigated the dietitian's role in this area.

Purpose: This study explored FS information provision to VP and the role of dietitians.

Methods: A mixed-methods approach included questionnaires completed by trainee-dietitians (*n*=34), VP (*n*=40 pregnant women, *n*=120 chemotherapy patients); review of FS resources available from NHS boards/trusts(*n*=159); and in-depth stakeholder interviews with the British Dietetic Association, dietetic academics, and registered dietitians.

Results: Overall, FS information provision for VP was lacking. Although all trainee-dietitians reported working with VP, only 35% recalled provision of FS advice, which was most frequently given to pregnant women. Less than half of pregnant women (45%) and chemotherapy patients (49%) reported receiving FS information. FS resources were available from 30% of NHS boards/trusts; considerable gaps existed and information varied greatly between sources. Interviews established that other food-related health issues (diabetes/obesity) were dietetic priorities. Not all VP meet with dietitians, however, dietitians are well-placed to facilitate cascade training to support other healthcare providers to inform and enable VP to mitigate foodborne illness risk. Currently, trainee-dietetic curriculum requires knowledge of FS legislation, often achieved by completion of FS programs designed for food industry employees. This content may not be clinically applicable/specifically tailored for dietitians delivering FS advice to VP. Indeed, 50% of trainee-dietitians believed the training they received was insufficient.

Significance: Amending trainee-dietician curriculum from knowledge of legislation to incorporate FS information provision, along with developing a continuing professional development program for registered dietitians, may improve dissemination of FS advice to VP and healthcare providers. Furthermore, standardized resources are required to support delivery of FS advice.

P2-110 Food-Safety Experts' Perceptions of the Potential of Television Cookery Programmes to Deliver Consumer Food-Safety Information

Simon Dawson[1], Ruth Fairchild[1], Nick Perham[2] and **ELLEN W. EVANS**[1]

[1]ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom, [2]Cardiff Metropolitan University, Cardiff, United Kingdom

❖ ***Developing Scientist Competitor***

Introduction: An estimated one million people suffer foodborne illness in the UK annually. Consumer food-safety malpractices in the domestic kitchen are documented as significant contributing factors to illness. Consequently, consumers need to be informed of adequate domestic practices to ensure food-safety. As a result, any resources that could influence domestic food-safety practices should be explored. The popularity of television cookery programmes, particularly those hosted by celebrity chefs, have increased in the UK. Many consumers' meal preparations are inspired/influenced by such programmes, consequently, there is a need to explore the potential of such television cookery programmes influencing domestic food-safety.

Purpose: To determine key domestic food-safety practices evaluated as important by food-safety professionals and explore the potential role of television food programmes in the delivery of food-safety/malpractice information.

Methods: An online questionnaire, distributed through online food-safety platforms, was completed by food-safety professionals (*n*=123) working in food manufacture (34%), lecturing (18%), technical consultancy (12%) and laboratories (11%).

Results: The most important food-safety practices for consumers were identified by food-safety experts as: decontamination of hands/surfaces, thorough cooking and safe refrigeration practices. Three quarters (75%) indicated television cookery programmes often included food-safety malpractices. Although 13-23% believed there was no place for food-safety information in television cookery programmes, as they are intended as entertainment only, the majority (75-76%) expressed positive attitudes towards cookery programmes being utilised to educate consumers regarding food-safety. All participants believed that the inclusion of adequate food-safety communication in cookery programmes could inform/influence/enable consumers to reduce the risks associated with foodborne illness in the home.

Significance: The research has determined the potential of television food programmes as a method to deliver food-safety information. However, given the lack of data regarding the food-safety content of UK television cookery programmes, there is a need to develop a tool to assess the adequacy of food-safety communication in cookery programmes.

P2-111 Assessing Recommendations Found in Recipes for Determining Doneness of Poultry: How Prevalent is Internal Temperature?

SANDRIA GODWIN[1], Edgar Chambers IV[2], Fur-Chi Chen[1] and Terry Taylor[2]

[1]Tennessee State University, Nashville, TN, [2]Kansas State University, Manhattan, KS

Introduction: Research has shown that many consumers do not follow recommended food safety practices for cooking poultry dishes, which can lead to foodborne illnesses such as Salmonellosis. For poultry, the USDA recommends cooking until the center of the thickest part of the meat reaches a minimum internal temperature of 165°F. In previous studies, consumers reported obtaining recipes from the internet, cookbooks, and family members.

Purpose: The purpose of this study was to assess instructions provided for determining doneness in recipes used for cooking poultry.

Methods: Using a structured instrument, 257 recipes for whole birds, parts, and ground patties cooked by various methods, including baked, fried, broiled, grilled, sautéed and slow cooked, were analyzed to assess recommended methods for determining doneness. A variety of resources, including cookbooks, websites, and magazines were included. All recipes had to begin with uncooked poultry.

Results: A specified end-point cooking temperature was given in only 23% of recipes. When a temperature was given, it was often incorrect; additionally, few instructions were given on where and how to insert the thermometer. Other indicators of doneness in the recipes included: internal color, including "no longer pink", external color, juices running clear, tenderness, crispiness of crust, minutes cooked, and the phrase "cooked through".

Significance: Consumers are not receiving information on using endpoint temperatures to determine the safety of cooked poultry from the recipes they follow. Some of the methods recommended such as color have been proven to be unreliable in recent studies. Recipes need to include appropriate end-point temperatures, which is the only safe means of ensuring safety. Further work is needed to determine the definition of cooked through.

P2-112 Impact of Poultry and Egg Education Project (PEEP) Workshops on Food Safety Knowledge, Perceptions, Attitudes, and Intentions of 4-H Youth

SANDRIA GODWIN[1], John Ricketts[1] and Morgan Beaty[2]

[1]Tennessee State University, Nashville, TN, [2]University of Tennessee, Lebanon, TN

Introduction: On average, one in six Americans becomes sick from foodborne illness each year. The risk is even higher among children and youth. According to the Centers for Disease Control. Two of the most common foodborne illnesses are caused by *Salmonella* and *Campylobacter*, which can be found in poultry and eggs. The Poultry and Egg Education Project (PEEP) aims to reduce instances of foodborne illness by educating consumers on food safety practices. Education through youth programming is one of the best ways to educate families and communities.

Purpose: This study evaluated knowledge gained, and the attitudes and intentions of implementation of lessons learned during an educational workshop presented to 4-H youth during summer camp that emphasized the safe handling and use of poultry and eggs.

Methods: Six lesson plans that addressed poultry and egg purchasing and handling, storage, preparation and personal and kitchen sanitation were organized into a one-hour workshop. A sample of 4th-6th grade 4-H youth attending various 4-H camps (n = 190), attended the workshop, during which they completed a pre-test, were taught the basic information in the lessons, and completed a post-test.

Results: There was a statistically significant difference between overall pre-test knowledge scores (M = 6.61, SD = 1.74) and post-test knowledge scores (M =10.46, SD =1.65); t(189)= -24.61, $P \leq .05$. Cohen's effect size value (d = 2.21) suggested a high effectiveness of the workshop in teaching poultry and egg safety themes.

Significance: The poultry and egg safe handling and use training for 4-H youth positively impacted knowledge, perceptions, and intentions. Findings from this study were used to revise and publish a full curriculum for youth on safe handling of poultry and eggs that is now available for educators and program leaders to implement.

P2-113 Determining the Presence of Pathogen Reduction Strategies at Livestock Interactions

SAVANA EVERHART[1], Derek Foster[1], Benjamin Chapman[1] and Megan Jacob[2]

[1]North Carolina State University, Raleigh, NC, [2]Department of Population Health and Pathobiology, CVM, NCSU, Raleigh, NC

Introduction: Outbreaks of *E. coli*, *Salmonella*, and *Cryptosporidium* have been associated with direct and indirect animal contact at petting zoos and agricultural fairs. Seven states have laws governing pathogen reduction strategies (primarily hand sanitation) be present at these exhibits. These laws do not apply to other livestock interactions such as agrotourism farms and zoos, however, outbreaks have been associated with public attendance at these venues.

Purpose: The purpose of our work was to host an interactive workshop for North Carolina agrotourism members, describe the types of interactions marketed, and determine the pathogen reduction strategies and facility design considerations targeted at reducing pathogen transmission in these venues.

Methods: An interactive workshop open to all North Carolinians operating or interested in pathogen control at livestock interactions was held in January of 2017. Participants (n = 44) were provide a questionnaire which collected information on current biosecurity and human public health practices, knowledge and concerns. Respondents (*n*=18) remained anonymous and were not provided specific recommendations prior to recording their results.

Results: All respondents reported that physical interactions (petting or touching) were allowed and encouraged in their events/sites. Sixteen operators indicated they had hand washing stations available to the public on their premise. Two operators reported that people often eat in the areas where animals are housed or on display. Only two operators reported any sort of cleaning and sanitizing of animal/human barriers. No operators reported having foot baths directly surrounding their interaction area.

Significance: Developing tools, including risk assessments, and educational material for livestock agrotourism operators that engage the public was requested and encouraged. Much variability in pathogen reduction knowledge and strategies was reported. Future work to identify critical risk points at livestock interaction exhibits is warranted to further reduce the outbreak potential at these venues.

P2-114 Animal Contact in Public Settings: Infectious Disease Risk Awareness and Hand Hygiene Behaviors

WENQING XU[1], Melissa Cater[2], Rebecca Gravois[2], Christine Navarre[3], Diana Coulon[3] and Dorra Simmons[1]

[1]School of Nutrition and Food Sciences, Louisiana State University AgCenter, Baton Rouge, LA, [2]Louisiana State University AgCenter, Department of Agricultural and Extension Education & Evaluation, Baton Rouge, LA, [3]School of Animal Sciences, Louisiana State University AgCenter, Baton Rouge, LA

Introduction: Enteric pathogens can be carried by healthy animals without them having any illness. From 2000 to 2014, animal-human interactions at state, regional, or county fairs; petting zoos; and educational farms have caused 30 gastroenteritis outbreaks in the United States. To reduce the risk of pathogens transferred through animal contact, people have to avoid certain high-risk behaviors and follow good hand washing practices.

Purpose: This study assessed the awareness of risk of animal contact in the public settings and assessed handwashing behavior among adults in Louisiana.

Methods: A questionnaire was designed to assess the participants' awareness of risk of animal contact and hand washing behavior after the animal contact in the state of Louisiana. The survey was conducted throughout the state in various settings including livestock shows, petting zoos, and educational farms. Data were analyzed using descriptive statistics.

Results: Two hundred and thirty five questionnaires were collected. Respondent demographic information indicated that participants were representative of the population of the State of Louisiana. The perception of risk associated with animal contact showed that the awareness towards risk behaviors, such as eating or drinking and nails biting, was poor. Most participants (76.2%) reported that they wash hands immediately after animal contact and 69.5% reported they sometimes use hand sanitizer in place of hand washing. A correlation study showed that past experience such as being sick after animal contact had positive correlations with awareness and handwashing behavior.

Significance: We found that adults in Louisiana had poor awareness towards the risk associated with animal contact in public settings. This revealed a need for increased communication and education for adults, especially parents, to reduce the risk of transferring enteric pathogen during animal contact.

P2-115 Identification of Prevention Efforts for Flour-associated Outbreaks Involving Shiga Toxin-producing *Escherichia coli*

Cerise Robinson, **TAMI CRAIG CLOYD**, India James, Marianne Fatica, Sheila Merriweather, Donald Obenhuber, Diane Gubernot and Gary Weber
FDA-CORE, College Park, MD

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) O121, O26, and O157:H7 are foodborne pathogens that can cause human illness, including bloody diarrhea and hemolytic uremic syndrome. From 2009 to 2016, these bacteria have been associated with four outbreaks involving flour and raw or undercooked dough products. Outreach by FDA regarding these outbreaks involved informing consumers and retailers via web postings.

Purpose: The purpose of this study was to analyze and trend the flour-associated STEC outbreaks, and identify effective prevention strategies.

Methods: FDA's Coordinated Outbreak Response and Evaluation Network (CORE) collected and compared surveillance information, epidemiological and laboratory data, and establishment inspectional information from the four outbreaks of flour-associated STEC infections. By analyzing this information, CORE identified similarities and differences among the outbreaks.

Results: Observed differences included the type of product (e.g. flour, raw cookie dough, and undercooked dough products) and routes of exposure. It was determined that contamination has the potential to occur in not only flour, but also in multi-ingredient flour products (i.e. dough products). The exposure routes in these outbreaks included consumption of raw cookie dough and undercooked dough products, as well as improper handling of raw dough at the consumer and retail food service levels. For all four outbreaks, FDA issued web postings notifying the public of the recalls and informing consumers and the food service industry about the risk of consuming or improperly handling flour, raw dough, and undercooked dough products.

Significance: These outbreaks provided evidence of STEC exposure via consumption or improper handling of flour, raw dough, and undercooked dough, and identified the need to further inform consumers and retail food services about safe handling of these products. Because the outbreak information suggests that educational outreach, consumer messaging, and updated product labeling may be the most effective means to prevent future illnesses, CORE will pursue discussions related to these potential strategies.

P2-116 Development of a FSMA Preventive Controls for Human Food Rule Audit Checklist for Fruit and Vegetable Processors

JACQUES OVERDIEP III, Angela Shaw, Catherine Strohbehn and Linda Naeve
Iowa State University, Ames, IA

❖ ***Developing Scientist Competitor***

Introduction: The Food Safety Modernization Act (FSMA) passed in 2011 created sweeping change throughout the food industry with a focus on control of food allergens and prevention of foodborne illnesses. FSMA Preventive Controls for Human Food rule was released September 2016 for facilities in manufacturing, processing, packing, and holding of human food.

Purpose: Develop an extension tool (audit checklist) for human food manufacturing facilities to determine if they comply with the FSMA Preventive Controls for Human Food Rule.

Methods: The audit checklist was developed by analyzing 21 CFR 117 for the requirements of the Human Food Rule, Subpart B (Current Good Manufacturing Practices) and Subpart C (Hazard Analysis and Risk-Based Preventive Controls), as well as the British Retail Consortium (BRC), and Safe Quality Foods (SQF) auditing schemes. It will be piloted by several companies before its public release to ensure all points are properly addressed and understandable.

Results: The 38-page finalized tool contains two checklists and is featured on the ncrfsma.org website. The first checklist is dedicated to Hazard Analysis and Risk-Based Preventive Controls (Subpart C), which includes 13 sections that walk through major aspects of Subpart C, whereas the second checklist contains 9 sections explaining features of Current Good Manufacturing Practices (Subpart B) such as sanitation, equipment, and personnel. Each section is split into several questions that describe how the plant should normally run as defined by 21 CFR 117. Hints were provided as further explanation for each point in the checklists while comments and definitions were added at the end of each section to emphasize important points and government definitions for cohesion and clarity.

Significance: FSMA implementation has been source of concern and confusion for many fruit and vegetable processors. These checklists will aid those processors in their compliance with the Human Food Rule.

P2-117 North Central Region Produce Needs Assessment for FSMA Produce Safety Rule

BRIDGET PERRY, Arlene Enderton, Catherine Strohbehn, Angela Shaw and Linda Naeve
Iowa State University, Ames, IA

❖ ***Developing Scientist Competitor***

Introduction: The increase in number of produce recalls and outbreaks due to contamination has resulted in focus toward industry practices and evidence-based education from farm to market.

Purpose: To assess knowledge and educational needs of produce growers in the north central region (NCR) of the U.S. for compliance with the Food Safety Modernization Act (FSMA) Produce Safety Rule.

Methods: A two-phase, modified Delphi approach was used to gather information from growers; Round One is presented here. Paper and electronic questionnaires were distributed by educators and organizations (*n*=30) in 12 Midwest states part of NCR. A five-point Likert rating scale was used to assess current knowledge and educational needs in areas of water testing; biological soil amendments; animal controls; worker health and hygiene; worker training; and equipment, tools, and buildings. A similar scale was used to identify preferred methods of information delivery. Space for comments was available. Comments were independently reviewed by four researchers with consensus reached on identified themes. The questionnaire was reviewed for content validity and clarity by NCR state partners.

Results: Findings indicated respondents (n = 299) were concerned about regulations, on farm best practices pre- and post-harvest, organic standards, and recordkeeping. The top four areas of information needs identified were water and biological soil amendments testing, training, and animal controls. Qualitative data identified concerns with understanding regulations and cost of compliance. Preferred information delivery methods were identified as in-person approaches and use of technology such as online modules and downloadable fact sheets. The use of social media had negative feedback with multiple comments noting this subject matter is too important for this type of technology.

Significance: Although developed for Midwest growers, this validated needs assessment could be used by food safety practitioners in other geographic regions to assess growers' educational needs and perceived best approaches in delivering information.

P2-118 Promoting Food Safety Research and Collaboration

JOHN JOHNSTON[1] and Glenn Tillman[2]
[1]*U.S. Department of Agriculture–FSIS, Fort Collins, CO,* [2]*U.S. Department of Agriculture-FSIS-OPHS, Athens, GA*

Introduction: The Food Safety and Inspection Service (FSIS) is the public health agency of the U.S. Department of Agriculture. FSIS is responsible for the safety of meat, poultry, egg products and Siluriformes (catfish). While FSIS is not a research organization, FSIS relies on research outcomes to guide policy and program direction.

Purpose: To identify and communicate opportunities for high impact food safety research, FSIS maintains an up to date list of research priorities. This list may be useful to researchers who are preparing grants for submission to agencies that fund food safety research, researchers with resources to conduct such research and/or research funding organizations. FSIS also promotes high impact food safety research through collaborations with food safety researchers

Methods: Research Priorities are communicated to the food safety research community via the FSIS website, presentations at scientific meetings and presentations/seminars at universities and other food safety research organizations. Collaborations may include sharing biological materials (e.g. microbial isolates, product samples) and data. Researchers interested in discussing FSIS Research Priorities and/or pursuing collaborative research should contact the FSIS Scientific Liaison (ScientificLiaison@fsis.usda.gov).

Results: Recent collaborations include identification of Shiga toxin producing E. coli in FSIS regulated beef, identification of food outbreak enabling phenotypic and genotypic traits of pathogen strains, development of techniques to reduce pathogen concentrations in livestock, determination of nitrosamines in cured bacon and determination of pharmacokinetics of perfluro environmental contaminants in steers.

Significance: The FSIS Research Priorities and collaboration process has spawned more than 60 collaborative research studies with university, government, and industry food safety researchers.

P2-119 Withdrawn

P2-120 Using Whole Genome Sequencing to Provide Insight in the Epidemiology of Resistance and Virulence Genes in *Listeria monocytogenes*

Katleen Vranckx[1], **KYLE KINGSLEY**[2], Koen Rombouts[1], Katrien De Bruyne[1] and Hannes Pouseele[1]
[1]*Applied Maths NV, Sint-Martens-Latem, Belgium,* [2]*Applied Maths Inc, Austin, TX*

Introduction: *Listeria monocytogenes* is a ubiquitous organism in the environment and a rare cause of human disease. Although its incidence is at least 100 times lower than those of other foodborne pathogens, such as *Campylobacter* or *Salmonella*, listeriosis is characterized by a high case-fatality rate which can exceed 30% percent in outbreak situations. Currently, every isolate in a food or clinical setting is considered problematic even though some isolates are more likely to persist in a food environment and/or cause human disease. Many virulence and resistance genes have been linked to these features, but no large scale investigation has been done on the presence of these factors in isolates from different environments. Therefore, our knowledge on the frequency and importance of known virulence and resistance genes in *L. monocytogenes* is limited.

Purpose: As more and more whole genome sequence data becomes available from surveillance, this data can be used to make an extensive study on the epidemiology of known resistance and virulence genes.

Methods: Publically available sequence read sets of over 10.000 *L. monocytogenes* isolates were assembled on the BioNumerics Calculation Engine using SPAdes. A reference database was created with all known virulence and resistance genes, as well as genes determining serovars. This database was used to screen all assembled genomes for the presence of these genes, and to predict the serovar.

Results: The BioNumerics® 7.6 software and its Calculation Engine offer a powerful platform on which WGS analysis can be performed and validated against traditional typing data, as well as phenotypic data. The genotyping tool provides the possibility to extract virulence- and antibiotic-related genomic signatures from WGS data.

Significance: The virulence genes and resistance genes could be easily extracted and compared to the available metadata, providing insight in the presence and distribution of these genes within all publicly available NGS data.

P2-121 Investigating the Epidemiology of Resistance and Virulence Genes in *Listeria monocytogenes* Using Bionumerics® 7.6

Katleen Vranckx[1], **KYLE KINGSLEY**[2], Katrien De Bruyne[1] and Hannes Pouseele[1]
[1]*Applied Maths NV, Sint-Martens-Latem, Belgium,* [2]*Applied Maths Inc., Austin, TX*

Introduction: *Listeria monocytogenes* (Lmo), although an uncommon cause of illness in the general population, is an important pathogen in pregnant patients, neonates, elderly individuals, and immunocompromised individuals. Following considerable cost reductions, complete Lmo genome sequencing has dramatically increased the number of publically available genomes on the Sequence Read Archive (SRA) of NCBI. Rapid and automated processing of whole genome sequencing (WGS) data ensures a reliable and easy to follow workflow in routine molecular surveillance, reducing the time needed to detect and contain an outbreak.

Purpose: In this study, we compared two subsequent pipelines for high resolution WGS-based molecular typing.

Methods: First, the BioNumerics® calculation engine was used to apply whole genome multilocus sequence typing (wgMLST) to WGS data from 10,000+ isolates, after which the alleles are downloaded to the BioNumerics®7.6 database and cluster analysis is performed. Second, clusters of closely related isolates from different food sources are further characterized by whole genome single-nucleotide polymorphism analysis (wgSNP) on the calculation engine. SNP variants are detected by mapping the WGS reads to a reference chosen from within the cluster to maximize the resolution.

Results: We demonstrate that wgMLST is suitable for the analysis of very large (and growing) datasets, making it an appropriate technique for outbreak surveillance. The added resolution of wgSNP against an internal reference sequence increases the confidence in the detected clusters and supports epidemiologists in their source tracking efforts.

Significance: This software platform performs high-throughput wgMLST and wgSNP analyses whose results can be validated against traditional data such as MLST or PFGE. The platform's integrated pipelines can provide robust, portable, high resolution and cost-effective molecular typing for food safety and public health monitoring programs.

P2-122 Storage Temperature and Sanitizer Washing Influences the Bacterial Community Dynamics of Carrots

VAISHALI DHARMARHA[1], Natalie Pulido[1], Giselle Kristi Guron[1], Monica Ponder[1], Amy Pruden[1], Renee Boyer[1] and Laura Strawn[2]
[1]*Virginia Tech, Blacksburg, VA,* [2]*Virginia Tech - Eastern Shore AREC, Painter, VA*

❖ ***Developing Scientist Competitor***

Introduction: Contamination of fresh produce by antibiotic-resistant bacteria (ARB) can occur at any point in the food chain. However, limited research is available regarding the effect of sanitizer washing and refrigerated storage on bacterial community dynamics, including ARB on fresh produce.

Purpose: This study analyzed changes to the bacterial community composition of carrots associated with different sanitizers and storage at recommended (2°C) and temperature abuse conditions (10°C).

Methods: Compost derived from manure, with and without prior antibiotic dosing to the dairy cows, was inoculated, separately, with multi-drug resistant *Escherichia coli* O157:H7 and *Pseudomonas aeruginosa*. To provide a background inoculum representing potential carryover of resistant bacteria from the field, carrots were dip-inoculated in compost slurry followed by air drying and a tap water wash with 50 ppm free chlorine or Tsunami 100 (40ppm peroxyacetic acid; 11.2% H_2O_2) per manufacturer's directions. Post drying, carrots were stored at 2°C or 10°C. DNA was extracted from the carrot surface and Illumina sequencing of 16SrDNA amplicons was performed.

Results: Carrots stored at 2°C had greater species richness than carrots stored at 10°C (P<0.0001, Shannon). Carrot bacterial communities inoculated without antibiotics containing compost were more even (Chao1) compared to compost with antibiotics (P=0.0190). Compost inoculated with ARB had more species evenness (Chao1) than non-inoculated compost (P=0.0191). OTUs belonging to 10 phyla were significantly affected by compost type, storage temperature, wash type, inoculation with ABR or a combination. Storage temperature significantly increased abundance of Pseudomonadales (P=0.0274). Carrot microbiota was mainly represented by the phyla Firmicutes, Proteobacteria, Actinobacteria, Planctomycetes, and Acidobacteria.

Significance: Refrigerated storage and use of a sanitizer wash offer strategies to alter the bacterial composition on carrots. Analysis of shot-gun metagenomics sequencing data is underway to identify the relationship between phylogeny and the resistome. This study can help inform management practices for limiting the dissemination of antimicrobial resistance from farm-to-fork.

P2-123 Complete Genome Sequence of a Novel Lytic *Vibrio parahaemolyticus* Phage VPp1 and Characterization of Its Endolysin

MENGZHE LI[1], Jingxue Wang[1], Hong Lin[1], Xiuping Jiang[2] and Yanqiu Jin[1]
[1]*Ocean University of China, Qingdao, China,* [2]*Clemson University, Clemson, SC*

Introduction: *Vibio parahaemolyticus* is a major pathogen causing foodborne disease. The application of bacteriophages and endolysins offers a new way to control the pathogen or cure bacterial infections with relatively high host specificity and low-resistant rates, which will do no harm to the human, animal hosts, and other beneficial bacteria.

Purpose: The objectives of this study were to sequence a novel lytic *V. parahaemolyticus* phage (VPp1) and determine the antibacterial activity of recombinant endolysin (LysVPp1) derived from this phage.

Methods: The phage DNA was extracted from a purified bacteriophage VPp1 and sequenced. Putative endolysin LysVPp1 (gp33) was predicted via nucleotide collection database by BLAST and amplified by PCR with phage VPp1 DNA as the template. The purified PCR products were cloned into vector pET-28a (+) and then transformed into competent cells Rosetta (DE3). Next, the transformants were induced with IPTG and purified. The antibacterial spectrum of bacteriophage VPp1 and LysVPp1 was determined by overlay method and gel diffusion assays, respectively, whereas the antibacterial activity of LysVPp1 was detected with the turbidity reduction method.

Results: The complete VPp1 genome sequence contained a circularly double-stranded DNA of 50,431bp with a total GC content of 41.35%. The genome was predicted to encode 67 gene products and two tRNAs. The average length of the gene is 752bp. In addition, LysVPp1 could lyse 9 out of 12 *V. parahaemolyticus* strains, showing its relatively broader host spectrum than 3 out of 12 of its preliminary bacteriophage VPp1. Furthermore, in the presence of LysVPp1, the optical density of bacterial cells treated with EDTA decreased by 0.4, showing hydrolysis properties for degrading the outer-membrane of cell envelope.

Significance: Our results lay a significant foundation for the development of novel enzybiotics for inhibiting *V. parahaemolyticus*.

P2-124 Whole Genome Sequence Analysis of Poultry-associated *Salmonella* Infantis Isolates from Turkey Reveal a Distinct Phylogenetic Clade and Increased Antimicrobial Resistance Elements

Sinem Acar[1], Ece Bulut[2], Yeşim Soyer[1] and **MATTHEW J. STASIEWICZ**[3]
[1]*Middle East Technical University, Food Engineering Department, Ankara, Turkey,* [2]*University of Nebraska at Lincoln, Department of Food Science and Technology, Lincoln, NE,* [3]*University of Illinois at Urbana-Champaign, Urbana, IL*

Introduction: *Salmonella* Infantis is emerging world-wide as an increasingly prevalent source of human salmonellosis and multi-drug resistant (MDR) *Salmonella* in the poultry supply chain. Previous research in Turkey used PFGE and antimicrobial resistance assays to find the clonally disseminated Infantis strains mainly had nalidixic acid-streptomycin-sulfonamide-tetracycline resistance type.

Purpose: The purpose of this study was to characterize MDR *Salmonella* Infantis in Turkey in comparison to all 242 *Salmonella* Infantis genomes deposited in the NCBI Sequence Read Archive as of May 2016.

Methods: We generated whole genome sequence data (Illumina MiSeq) on 23 *Salmonella* Infantis from Turkey and exclusively used open-source tools and public databases for phylogenetics and antimicrobial resistance analysis.

Results: Phylogenetic analysis of 46,000 Single Nucleotide Polymorphism (SNPs) across the core and accessory genome revealed the Turkish isolates clustered in a single, well-supported clade. The clade did not contain any comparison genomes. *In silico* screening identified 5 AMR genes that were present in all 23 Turkish isolates: *aac6*, *aadA1*, *sul1*, *tetA* and *tetR*, which were consistent with the previous research. While *aac6* was present in nearly all comparison isolates, prevalence of 4 other genes was significantly (P<0.001) higher in the Turkish isolates. Prevalence of AMR genes in comparison isolates were: *aac6*: 240/242(99%); *aadA1*: 46/242(19%); *sul1*: 66/242(27%); *tetA*: 72/242(30%) and *tetR*: 71/242(29%). Two resistance genes *qnrS* (quinolone) and *sul3* (sulfonamide) were identified in Turkish *Salmonella* isolates for the first time.

Significance: These results suggests these isolates from Turkey represent a single, endemic population of *S.* Infantis in Turkey, rather than a population repeatedly introduced from elsewhere. In addition, the prevalence of multi-drug antimicrobial resistance among these isolates from Turkey is higher than among other *S.* Infantis. Finally, the use of open-source tools and public databases show how existing bioinformatics resources can be leveraged to ask applied food safety questions relevant to international food systems.

Poster

P2-125 Evaluation of Whole Genome Sequencing (WGS) to Molecularly Characterize, Serotype, and Predict Antibiograms of *Salmonella* spp. Isolated from Raw Chicken Products in Singapore

YE HTUT ZWE[1], Seow Fong Chin[2], Liang Yang[2] and Hyun-Gyun Yuk[3]

[1]*Food Science and Technology Programme, National University of Singapore, Singapore, Singapore,* [2]*Singapore Centre for Environmental Life Sciences Engineering, School of Biological Sciences, Nanyang Technological University, Singapore, Singapore,* [3]*Korea National University of Transportation, Chungju-si, South Korea*

Introduction: Salmonellosis is the most common foodborne disease in Singapore, comprising up to 75.6% of all reported foodborne diseases in 2014. It is important to be able to rapidly characterize, perform source attribution, and predict the antibiotic resistance for tracking and clinical decision making in cases of outbreaks.

Purpose: This study aimed to compare the serotype, genotype, and predicted antibiograms obtained through WGS to those of traditional methods such as multilocus sequence typing (MLST), serotyping, and disk diffusion antibiotic susceptibility testing; thereby, assessing the potential of WGS to replace these traditional methods as a faster, more reliable characterization system for an outbreak investigation.

Methods: Using the ISO method, a total of 42 *Salmonella* spp. strains were isolated from retail raw chicken products being sold in Singapore. The sequence types and serotypes of the isolates were determined by MLST. Their antibiotic resistances were examined by the disk diffusion method. Subsequently, the whole genome of each isolate was sequenced using an Illumina system. Using the whole genome sequence of each isolate, the presence of the antibiotic resistance genes was determined using the ResFinder 3.0.

Results: WGS pipelines were able to accurately determine the MLST sequence types and serotypes of the 42 isolates with 100% accuracy. Overall, the antibiotic resistance profile from WGS could predict the phenotype with 98.5% sensitivity and 56.3% specificity. The specificity was the lowest for aminoglycosides (11.8%) and quinolones (33.3%). Single nucleotide polymorphism (SNP) trees obtained from WGS data could further genotypically characterize *S.* Saintpaul (n = 17) and *S.* Brancaster (n = 11) isolates, which were not distinguishable by MLST.

Significance: This study indicates that WGS could be an effective method to obtain accurate sequence types and serotypes of *Salmonella* spp. without the hassles of laborious MLST and serotyping procedures. Molecular typing by SNP trees through WGS could offer superior resolution as compared to the traditional MLST.

P2-126 Genetic and Phenotypic Characteristics Associated with *Listeria monocytogenes* Plasmids

PATRICIA HINGSTON[1], Jessica Chen[2], Chad Laing[3], Lisbeth Truelstrup Hansen[4] and Siyun Wang[1]

[1]*University of British Columbia, Vancouver, BC, Canada,* [2]*IHRC, Inc., Decatur, GA,* [3]*Public Health Agency of Canada, Lethbridge, AB, Canada,* [4]*Technical University of Denmark, Kgs. Lyngby, Denmark*

❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* (*Lm*) strains are known to harbor plasmids, with an average prevalence of 30%. While *Lm* plasmids have been shown to confer resistance to certain sanitizers, heavy metals, or antibiotics, it is unknown whether they facilitate resistance to common food-related stresses.

Purpose: The objectives of this study were to compare the genes found on different *Lm* plasmids and determine if plasmid harborage is associated with enhanced resistance to food-related stresses.

Methods: Whole genome sequencing was performed on 166 *Lm* strains and the assemblies were screened for the plasmid replicon gene, *repA*. All sequences of *repA* positive strains were aligned to the closed genome of *Lm* EDG-e using Mauve. Contigs not aligning to EGD-e were excluded as plasmid-associated if they contained chromosomal DNA elements or did not align to any previously published plasmids. The resulting concatenated plasmid sequences were divided into two groups (G1, G2) using a *repA* phylogeny. Additionally, plasmid-positive and -negative strains were compared on their ability to tolerate cold (4°C), salt (6% NaCl), acid (pH 5), or desiccation stress (33% RH).

Results: Plasmids were observed in 55% of strains and plasmid-harborage was associated (P=0.013) with enhanced acid tolerance. G1 plasmids were significantly smaller (26-88 kb) than G2 plasmids (P<0.0005, 55-100 kb). The most prevalent plasmid (56,553 bp – G1) was observed in 26 strains from both Switzerland and Canada. G1 plasmids uniquely contained genes encoding alcohol dehydrogenases, and specific lipoproteins, while G2 plasmids contained genes for DNA topoisomerase III, DEAD box helicases, thermonucleases, and multidrug resistance proteins (*ebrAB*). Noteworthy, one strain, which exhibited the highest level of acid tolerance, contained two plasmids (i.e., one from each *repA* group).

Significance: This work highlights the widespread abundance of specific *Lm* plasmids, the unique properties associated with G1 and G2 plasmids, and the association between plasmid harborage and enhanced acid tolerance.

P2-127 A Novel Method to Achieve Complete Low-copy Number Plasmid Sequences of *Salmonella enterica*

KUAN YAO[1], Narjol Gonzalez-Escalona[1], Julien Marquis[2], Marc Allard[1] and Maria Hoffmann[1]

[1]*U.S. Food and Drug Administration, College Park, MD,* [2]*Nestlé Institute of Health Sciences, Lausanne, Switzerland*

Introduction: Plasmids play a major role in bacterial adaption to environmental stress and often contribute to antibiotic resistance and disease virulence. To study plasmid biology the complete plasmid sequence is essential. The majorities of antibiotic resistance and virulence plasmids in *Salmonella* have a low copy number and unfortunately, are hard to extract and to sequence. The existent protocol consists in a laborious transformation of the plasmid solution, extracted from *Salmonella*, into *Escherichia coli* in order to achieve enough DNA for accurate sequencing. Therefore there is a need for a fast method to produce high quantity and good quality of plasmid DNA.

Purpose: The purpose of this study was to test the efficiency of rolling circle amplification for replicating *Salmonella* plasmid DNA to a satisfactory amount making it suitable for sequencing.

Methods: Six *Salmonella enterica* isolates, carrying known low copy number plasmids, representing five different serovars were cultured and the plasmids were isolated using the Qiagen Plasmid Mini Kit. The plasmid DNA solution was amplified using the REPLI-g Mitochondrial DNA kit. The amplified plasmids were sequenced on the Pacific Biosciences *RSII* sequencer.

Results: High copies of *Salmonella* plasmid DNA were acquired through rolling circle amplification for sequencing. We were able to completely close all five *Salmonella* plasmids (size ranged from 38 Kb to 190 Kb) with a sequencing coverage from 2,400X to 5,800X. All *de novo* assembled plasmids aligned 100% with their reference genome on NCBI.

Significance: This novel protocol, consisting of plasmid isolation, replication and sequencing, is a valuable tool for closing high-molecular weight and low-copy-number plasmids. The rolling circle amplification was proven to be an effective and fast method for plasmid DNA replication by passing the low copy number hindrance without laborious transformation into *Escherichia coli*. This protocol will be beneficial for high throughout plasmid sequencing.

Poster

P2-128 Phylogenomic Analyses of Efflux Pump Complexes in the Foodborne Pathogen *Cronobacter* spp. Using DNA Microarray Analysis Combined with Sequenced-based Bioinformatics Demonstrates the Presence of Species-Specific Orthologues.

FLAVIA NEGRETE, Jayanthi Gangiredla, Samantha Finkelstein, Isha Patel, Hannah Chase, ChaeYoon Lee, HyeJin Jeong, Ben Tall and Gopal Gopinath
U.S. Food and Drug Administration, Laurel, MD

Introduction: *Cronobacter* spp. cause disease in humans of all ages, but is most noted for causing infantile foodborne outbreaks. Microorganisms express a wide range of transmembrane complexes known as efflux pumps (EPs) that influence their survival under stressful growth conditions. Additionally, through active efflux EPs are also involved in antimicrobial resistance to a variety of antibiotics, disinfectants, and preservatives. To date very little is known about EPs among *Cronobacter*spp.

Purpose: This project employed both microarray analysis (MA) and whole genomic sequencing (WGS) in an effort to provide a deeper understanding of the phylogeny of EPs found among *Cronobacter*spp.

Methods: One hundred thirty-nine *Cronobacter* strains were analyzed using a previously described pan-genomic DNA microarray which contained 37 EP probe sets representing 13 different EPs. Using the microarray annotations for these genes, gene sequences for three of these EPs, namely, *kefA* (a potassium efflux system), a resistance/nodulation/division (RND) EP gene, and *cmeB* (a tranporter) were bioinformatically captured by searching a local database of previously sequenced *Cronobacter*genomes. Phylogenetic analysis of these sequences was conducted using the Maximum Composite Likelihood method in Genomics CLC and MEGA7.

Results: MA revealed that the prevalence of the 37 efflux pumps followed evolutionary species lines which were previously noted. WGS analysis of sequences for *kefA,* the RND EP, and *cmeB*, supported this phylogenetic relationship found by MA.

Significance: Understanding the genomic landscape of EPs among *Cronobacter* spp. is essential for future studies designed to identify mechanisms used by *Cronobacter* spp. to survive and persist under stressful growth conditions. The results reported here demonstrate that *Cronobacter* species have at least 37 efflux pumps and that all of the species possess species-specific orthologues; that these genes most probably arose from a common hypothetical ancestor; and their identification is critical for reliable prediction of gene function.

P2-129 Development of a New Generation Microarray Assay for the Detection and Identification of Foodborne Pathogens

CHRISTINE YU[1], Mark Mammel[2], Jayanthi Gangiredla[1] and Michael Kulka[1]
[1]U.S. Food and Drug Administration, Laurel, MD, [2]U.S. Food and Drug Administration–CFSAN, Laurel, MD

Introduction:

The detection and identification of microbial contaminants in food are essential for prevention and investigation of foodborne illnesses. There is increasing demand to develop methods for the rapid and reliable detection of foodborne pathogens. Currently, nucleic acid-based detection remains the method of choice. Microarray analysis, one such nucleic acid-based detection, has been applied to simultaneously detect and genotype multiple foodborne pathogens.

Purpose:

The purpose of this study was to develop a new generation custom DNA microarray for detecting and identifying foodborne pathogens from multiple sources of samples. The new array is aimed to achieve better sensitivity and broader pathogen coverage than the previous tiling array with Affymetrix GeneChip design.

Methods:

The new, high-density peg format design is based on individual gene sets of selected virus strain containing a central nucleotide mismatch for paired-complimentary probe set. In addition to common foodborne viruses, the design of the new array specifically increased the sequence density for human norovirus and selected surrogates detection, as well as internal (RNA) controls for food processing.

Hepatitis A virus (HAV) strain HM175/18f and norovirus (NoV) strain MD145 are used as viral targets for protocol optimization and performance evaluation. Viral RNA is either extracted from culture supernatant or synthesized by in vitro transcription. Microarray analysis is performed following the modified Affymetrix GeneAtlas protocol.

Results:

The new microarray can detect and identify HAV HM175/18f as a subtype IB strain and NoV MD145 as GII.4 genogroup. Multiple approaches to data analysis are being assessed. Assay specificity was confirmed by the complete absence of cross reactivity observed between and/or among unrelated viral species.

Significance:

We demonstrate the development and application of a new custom DNA microarray for detection of foodborne pathogens. This method is being developed in order to address multi-virus detection and identification in FDA surveillance and outbreak investigations.

Poster

P2-130 Utility of the FDA-ECID Microarray for Comprehensive Molecular Serotyping of *Escherichia coli*

KYSON CHOU[1], Isha Patel[2], Jayanthi Gangiredla[2], Nelly Tran[1], Donna Williams-Hill[1], Richelle Richter[1], Peter Feng[3], Keith Lampel[2] and Christopher Elkins[2]
[1]U.S. Food and Drug Administration, Irvine, CA, [2]U.S. Food and Drug Administration, Laurel, MD, [3]U.S. Food and Drug Administration, College Park, MD

Introduction: With expanding serogroups of Shiga toxin-producing *Escherichia coli* (STEC), including novel hybrid pathotypes, increasingly causing foodborne illness, serotyping *E. coli* has become important. *Escherichia coli* is serologically complex. Over half of the *E. coli* isolated from foods are untypeable or only yield partial serotypes. A means to quickly determine the serotype of STEC would be useful in surveillance programs to reduce the distribution and consumption of contaminated foods. Serotype data is also critical in epidemiology and outbreak investigations, where it can expedite product recall and minimize outbreak impact.

Purpose: Following the guidelines of the FDA Method Validation Subcommittee, the current study aimed to validate the FDA-ECID microarray, as a rapid means for robust genetic serotyping of *E. coli* isolates.

Methods: Genomic DNA from a panel of reference *E. coli* strains were assayed in triplicate on the FDA-ECID microarray. Each strain was analyzed to determine both the O and H determinants, as well as the STEC virulence genes that may be indicative of potential for severe health risks. The array typing results were compared with the known profiles of the corresponding strains.

Results: A hierarchical cluster dendrogram, based on differences in probeset intensities, suggested only 91.8% reproducibility amongst the replicates. To ensure accurate identification of isolates analyzed, modified standard operating procedure for analysis and quality control were developed

and implemented. Analysis using quality controlled results showed that for all 85 isolates analyzed, replicates of each isolate clustered together in the dendrogram and had the same serotype that matched the known profiles of the corresponding strains; thus, demonstrating the reliability and validity of the FDA-ECID microarray for genetic serotyping.

Significance: Validation of the FDA-ECID microarray provides another efficient and effective tool for the Agency to use in identifying *E. coli* and facilitating health risk assessments of pathogenic *E. coli* in foods.

P2-131 Phylogenomic Analyses of Type II Toxin-Antitoxin Genes in the Foodborne Pathogen *Cronobacter* spp. Using Sequenced-based Bioinformatics Combined with DNA Microarray Analysis Demonstrates an Evolutionary Shared Species-Specific Line of Evolution

SAMANTHA FINKELSTEIN, Jayanthi Gangiredla, Flavia Negrete, Hannah Chase, ChaeYoon Lee, HyeJin Jeong, Isha Patel, Gopal Gopinath and Ben Tall

U.S. Food and Drug Administration, Laurel, MD

Introduction: *Cronobacter* spp. an etiologic agent of infantile foodborne outbreaks can survive extreme desiccation conditions, thus favoring its persistence in powdered infant formula and dry environments. Expression of toxin-antitoxin type II (TA) systems is thought to modulate bacterial physiology and survival during periods of stressful growth conditions. The phylogenetic landscape of TA loci among *Cronobacter* spp. is poorly understood.

Purpose: This investigation was conducted to provide a deeper understanding of the phylogeny of TAs found among *Cronobacter* spp.

Methods: One hundred forty-three WGS assemblies were uploaded to the web-based TAfinder tool at http://202.120.12.135/TADB2/index.php to identify TA loci. Additional analyses were conducted using a pan-genomic DNA microarray (MA). Sequences of these loci were then used to confirm presence of TA alleles on the microarray and in phylogenetic analysis using CLC Genomics workbench and MEGA7.

Results: On average approximately six TAs such as *relE-xre*, COG5654-*xre*, *fic-phd*, *fic-yhfG,* and *hipA* loci with BLAST scores of >90% were found. Fifty-five TA alleles were confirmed to be present on the microarray which represented six of the seven *Cronobacter* spp. Twenty-six duplicated alleles were removed from MA which showed that in some, but not all species, the TA allelic sequence divergence aligned with species taxa lines. For example, MA showed that 10 *C. malonaticus* strains clustered with some *C. sakazakii* strains signifying that sequence divergence of some TAs between these two species share a common phylogenetic history. WGS analysis of *fic*-like and a *hipA*-like orthologues of the seven species supported the phylogenetic relationship found by MA.

Significance: Understanding TAs among *Cronobacter* spp. is essential to design future studies identifying mechanisms used for survival and persistence in foods. These results demonstrate that in some species, TA alleles share a common phylogeny, while in other species, they follow species-specific phylogeny. Their identification is critical for reliable prediction of gene function.

P2-132 Interlab Comparison of Community Analysis Via Next Generation Sequencing

MATTHEW MARKIEWICZ[1], Clyde Manuel[2] and Stephen Lyon[1]

[1]*Sealed Air Corporation, Sturtevant, WI,* [2]*Sealed Air Corporation, Milwaukee, WI*

Introduction: Next generation sequencing (NGS) enables qualitative analysis of the composition of microbial communities. However, the method can be fairly sensitive to sample preparation and analytical techniques.

Purpose: The purpose of this study was to investigate NGS as a reliable method for qualitative community analysis and to characterize interlab variability.

Methods: Three independent laboratories were contracted to conduct NGS-based bacterial community analysis of two samples (in triplicate). The first sample was prepared in-house and represented a known composition of *Mycobacterium smegmatis* (73.1%)*, Pseudomonas aeruginosa* (24.3%)*, Escherichia coli* (1.2%), *Staphylococcus aureus* (1.0%), and *Bacillus subtilis* (0.3%). The second sample consisted of an environmental swab taken from a kitchen floor which represented a sample of unknown composition. Labs were instructed to perform bacterial community analysis using their own methods and procedures. The results from each lab were compared for accuracy and consistency across replicates, as well as consistency across labs.

Results: For known samples, each lab failed to identify the presence of *M. smegmatis*. The remaining species were detected at varying levels, for example, *B. subtilis* was detected at levels ranging from 0.3-17.3%. For field samples, each lab was in agreement on the top four contributors to the community, with *Psychrobacter* spp. (16.5-48.5%) and *Enterobacteriaceae* (14.2-25.5%) being the most abundant organisms. Large variations arose after the top four organisms, for example, one lab identified *Rothia* spp. being present at 7.5% while other labs did not detect the organism.

Significance: NGS may provide insight into community membership, but there is considerable variability in the results from identical samples. The results of the study highlight interlab variability while demonstrating the need to harmonize sample preparation and analytical techniques used in NGS.

P2-133 Leveraging Microbiome Analysis to Discriminate between Organic and Non-organic Produce: Apple Case Study

KENNETH HARARY, Abhishek Hegde, Hossein Namazi, James Maloney, Shadi Shokralla, Anay Campos and Ramin Khaksar

Clear Labs Inc., Menlo Park, CA

Introduction: With the rise of organic products, authentication becomes important as economically-motivated incidents become more of a risk. Combining the large capacity and robustness of Next Generation Sequencing (NGS) and the accuracy and reliability of barcoding, this study compares the microbial maps of the communities that thrive on the surface of organic and non-organic apples and examines the potential of these maps to be used as an authenticity test for organic products.

Purpose: The objective of the study is to measure the microbial differences between different varieties of organic and non-organic apples.

Methods: 16 organic varieties and 14 non-organic varieties of apples were purchased, and the skins of the apples were sampled for mass DNA extraction. Universally accepted regions, with markers covering 85% of ribosomal 16s region, were amplified in a 96-well format, using multiplex Polymerase Chain Reaction (PCR). PCR products were sequenced on the MiSeq platform to identify the species present in the apples.

Results: A signature profile of the microbial communities between the two categories of apples was established, and a clear trend emerged. The bacterial signatures were quite distinct, with Sphingomonas being the only commonality between organic and non-organic apples. Mycobiome signatures also differed significantly; several genera, such as Comoclathris and Exophiala, were detected only in non-organic products. Further, more species of microorganisms were found in non-organic than organic.

Significance: As the discussion of how different foods' microbiomes affect consumers becomes more pervasive, creating these signature profiles for organic and non-organic produce will be essential in understanding how certain products, pesticides, and foods are linked to public health. Moreover, consumer access to microbiome and mycobiome data may aid in making confident and healthier dietary choices.

P2-134 Short-term Supplementation of Potato Starch and VSL#3 in Male C57BL/6 Mice

MIGUEL RIVAS, Nadia Saadat, Liyanage Nirasha Perera, Smiti Gupta and Yifan Zhang
Wayne State University, Detroit, MI

❖ ***Developing Scientist Competitor***

Introduction: The gut microbiome may play a role in the development of chronic diseases like obesity, diabetes, and heart disease. Diets including prebiotics or probiotics can alter the abundance of gut bacterial groups, which may have subsequent health effects.

Purpose: *I*n this study we wanted to monitor fecal microbial profiles of 21-day-old male C57BL/6 mice (*n*=24) exposed to 15 days of dietary supplementation.

Methods: A probiotic diet (VSL#3) and a prebiotic diet (potato starch) were compared to a standard diet (*n*=8 for each group). Body weight, food, and water intake were monitored periodically. Fecal microbial profiles were obtained through qPCR using group-specific 16S RNA primers (total bacteria, enterobacteria, bifidobacteria, lactobacillus).

Results: The potato starch group showed higher body weight than the controls ($P<0.05$), but was similar to the VSL#3 group. No difference in weight was observed between the VSL#3 group and the controls. The large intestine weight of the potato starch group was higher than the controls and the potato starch group ($P<0.05$). The qPCR data showed that 500 pg was the optimal DNA loading amount for the standards (*Escherichia coli, Bifidobacterium infantis, Lactobacillus rhamnosus*), as well as fecal samples. Annealing temperatures of 55-60°C worked best for amplification. Food intake remained the same across the groups. Daily water consumption was higher in the VSL#3 group (6.65 ± 1.38 mL) as opposed to the potato starch and the control group (5.55 ± 1.21 mL, 5.54 ± 0.77 mL, respectively).

Significance: This study showed that VSL#3 and potato starch are suitable to use in a mouse model to study their impact on the gut microbiome. The probiotic water mixture using VSL#3 is a good method for incorporating beneficial bacteria in the diet without the need of invasive techniques like oral gavage.

P2-135 Microbial Ecology Survey of Bacteria, Lactic Acid Bacteria, and Fungi in Fermented and Non-fermented Ready-to-Eat Food and Drink

MATT HUNDT, Alexandra Smith and Tom Rehberger
Agro BioSciences Inc, Wauwatosa, WI

Introduction: In fermented foods, starter cultures are added or natural populations are encouraged to grow; while in other foods microbes are present and are only considered problematic if they grow and spoil the food, produce toxins, or are inherently pathogenic to consumers. With the advent of next generation sequencing (NGS), it is now possible to determine the total microbiota of foods without having to culture specific bacteria on selective agar.

Purpose: NGS of marker genes for total bacteria, lactic acid bacteria, and fungi was performed to determine the variety of microbes present in fermented and non-fermented food types across different manufacturers.

Methods: DNA was extracted from samples of fermented cottage cheese, kombucha, and sour ales, as well as non-fermented guacamole and hummus. Samples were obtained from three manufacturers per type of food. PCR was performed with 16S V4 primers for total bacteria, 16S primers selective for lactic acid bacteria, and fungal ITS primers using the Fluidigm Access Array technology. Illumina sequencing was performed on pooled amplicons and sequence analyses were performed using QIIME v 1.9.

Results: Most sequences obtained for hummus and guacamole were chloroplast and mitochondrial sequences from the plants, herbs, and spices in the foods. *Lactococcus lactis* was predominant in cottage cheese, while *Lactobacillus* species and *Pediococcus damnosus* were present in sour ales. *Brettanomyces bruxellensis* was the predominant yeast in two of the three breweries, while the majority in the other was *Saccharomyces cerevisiae*. The greatest diversity and variability within and between manufacturers was found in the kombucha samples for bacterial, LAB, and fungal sequences.

Significance: Microbial ecology was distinct by food type, with the predominant microbes in fermented foods being those of starter cultures, both bacterial and fungal. Further work is needed to determine whether lower chloroplast levels in non-fermented plant-based foods could be an early indicator of spoilage.

P2-136 Food Microbiomes Defined Using 16S rRNA Gene Amplicon and Shotgun Metagenomic Sequencing

KAREN JARVIS[1], Ninalynn Daquigan[2], Christopher Grim[3], James White[4], Paul Morin[5], Julia Mullins[6] and Darcy Hanes[3]
[1]U. S. Food and Drug Administration, Laurel, MD, [2]U.S. Food and Drug Administration, Laurel, MD, [3]U.S. Food and Drug Administration, CFSAN, Laurel, MD, [4]Resphera Biosciences, Baltimore, MD, [5]U.S. Food and Drug Administration, Jamaica, NY, [6]U.S. Food and Drug Administration–ORA, Jamaica, NY

Introduction: Foodborne pathogen culture methods vary by food matrix and pathogen of interest. Most require a nonselective pre-enrichment step to resuscitate injured pathogens followed by selective enrichment to reduce background microorganisms.

Purpose: The purpose of this study was to use shotgun metagenomic and 16S rRNA amplicon sequencing to identify the microbial communities in foods suspected of adulteration.

Methods: Eight cilantro samples (distributor or retail), five masala spice mixes (imported), seven cucumbers (retail), and two brands of mung bean sprouts (retail) were sequenced. 16S rRNA gene amplicon and shotgun metagenomic data were analyzed with Resphera Insights and COSMOSID, respectively.

Results: Enterobacteriaceae were significantly higher in cucumber, masala, and sprouts relative to cilantro ($P<0.002$; Mann-Whitney test), with sprouts maintaining the highest relative abundance (mean 55.2%). Observed species were the highest in the masala (~983) and lowest in mung bean sprouts (~277). Sprout brands were further distinguished by a predominance of spoilage associated *Leuconostoc* (54%) and *Lactococcus* (29%) species in brand A. *Flavobacterium* species were only present in cilantro; abundances ranged from 7% to 34%, depending on the source. The cucumber samples had approximately 340 observed species, with a high variability in abundance distributed among individual samples. For example, the average proportional abundance of *Rhizobium* was 12%; however, one cucumber sample contained a 50% proportional abundance and, *Paenibacillus* ranged from 5% to 31%. Lastly, three of the masala spice mixes were contaminated with *Plautia stali*, a bacterial symbiont specific to "stink" bugs, at proportional abundances ranging from 1.5 to 3.2% suggesting contamination with filth.

Significance: Metagenomic methods can identify the bacteria harbored in foods to improve culture methods and food quality. Our data reveal the diversity of microbial communities in different commodity types and sources such as, *Paenibacillus* that can hinder pathogen detection, spoilage bacteria, and food adulterants such as *P. stali*.

P2-137 Microbiota of Retail Foods Available to Populations of Different Socioeconomic Status: Implications to Food Safety

DALENIECE HIGGINS[1], Chandan Pal[2], Irshad Sulaiman[3] and Pratik Banerjee[1]
[1]*University of Memphis, Memphis, TN,* [2]*University of Gothenburg, Gothenburg, Sweden,* [3]*U.S. Food and Drug Administration, Atlanta, GA*

❖ ***Developing Scientist Competitor***

Introduction: The microbial community structure of retail foods available in areas with higher food insecurity and low socioeconomic status (SES) may differ significantly from high SES areas. Since the microbial ecology of foods may be associated with potential presence of pathogens, a comprehensive evaluation of the microbiota of foods sold at retail stores from different SES areas is important to understand potential risk of foodborne illness resulting from consumption of such foods.

Purpose: The purpose of our study was to evaluate the overall bacterial diversity, as well as the prevalence of antibiotic resistance genes (ARGs) in bacterial communities of foods sold at retail outlets in low and high SES areas in Memphis-Shelby County, Tennessee.

Methods: We utilized next generation sequencing of the 16S rRNA genes, shotgun metagenomics, PCR, as well as culture-dependent methods to evaluate the bacterial community of selected foods (lettuce, cabbage, deli meat, and chicken legs; *n*=200) procured from retail outlets.

Results: Foods from low SES areas contained higher bacterial counts and a different microbial composition compared to high SES areas. Firmicutes and Proteobateria were the most abundant phyla in produce and animal products. *Enterobacter* and *Pantoea* in produce, and *Bacillus* and *Aeromonas* in animal products were the most abundant genera. The prevalence and abundance of ARGs in bacterial communities of foods sold by retailers from different SES areas varied significantly. The highest abundance of ARGs was found in low SES deli meat, including ARGs to carbapenem and multidrug efflux systems. ARGs to amphenicols and erythromycin were present in all high SES lettuce and deli meats, respectively. The class 1 integron-integrase genes were detected in all chicken samples.

Significance: Consumption of foods containing substantially different microbiota and ARGs may expose populations of low SES to a differential risk of contracting foodborne illness than that of high SES.

P2-138 Foodborne Outbreak Detection: Florida Department of Agriculture and Consumer Services' WGS SNP Pipeline in Action

S. Brian Caudle, **CARL FRANCONI, JR.**, Serena Giovinazzi, Amy Bryant and Jason Crowe
Florida Department of Agriculture and Consumer Services, Tallahassee, FL

Introduction: Whole Genome Sequencing (WGS), together with its applications, is revolutionizing food safety. WGS provides a depth, specificity, and sensitivity previously unseen in outbreak detection technology by allowing isolate relationships to be elucidated by evolutionary descent. Due to the computational complexity required for WGS analysis, data interpretation is an obstacle for state labs, who are reliant on federal analysis pipelines.

Purpose: FDACS Bureau of Food Laboratories (BFL) has applied current technologies to develop an internal genomics pipeline to pinpoint possible foodborne outbreaks. This study demonstrates BFL's ability to compare genomic data from clinical, food, and environmental *Listeria monocytogenes* isolates to deduce SNP-based relationships.

Methods: FL DOH genomic data from eight clinical *L. monocytogenes* isolates was compared against BFL's *L. monocytogenes* phylogenic tree, containing approximately 700 Florida food and environmental isolates. Internal isolates were sequenced on a MiSeq using the Nextera XT and MiSeq V2 500-Cycle chemistry kits. An in-house developed pipeline consisting of kSNP3, FastTree2, and FigTree was used to establish preliminary sequence comparisons. The internally applied CFSAN SNP pipeline was used to detect hqSNPs amongst the cluster of interest. Lastly, FastTree2 was reincorporated along with FigTree to identify SNP distances and produce a phylogenetic tree.

Results: A cluster containing food and environmental isolates was found to be the closest to seven of the eight clinical samples. However, hqSNP analysis for this group showed that there were between 6,400 and 6,500 SNPs separating the clinical and food and environmental isolates. There were 1 to 4 SNPs identified within the cluster of seven clinical isolates.

Significance: BFL's usage of an in-house SNP pipeline quickly disproved a possible correlation eight clinical *L. monocytogenes* isolates had to current food and environmental isolates within Florida. This highlights the BFL's capability to promptly identify in-state foodborne outbreaks using a high resolution hqSNP-based approach.

P2-139 Characterization of the Young Turkey Cecal Microbiome and Its Role in the Prevention of Irritable Crabby Syndrome (ICS)

MARGARET KIRCHNER[1], Donna Carver[1], Brian Badgley[2] and Sophia Kathariou[1]
[1]*North Carolina State University, Raleigh, NC,* [2]*Virginia Tech, Blacksburg, VA*

❖ ***Developing Scientist Competitor***

Introduction: Turkey production is vital to the US poultry industry. However, young turkeys are susceptible to a specific syndrome, designated Irritable Crabby Syndrome (ICS), which leads to gastrointestinal distress, weight loss, and failure to thrive. ICS is currently treated with a combination of penicillin and gentamicin; but, with increasing public concern about antibiotic use in animal production, interest in promoting a healthy gut microbiota, as a preventative treatment, has increased. Impacts of ICS and accompanying antimicrobial treatment on the gut microbiome remain uncharacterized.

Purpose: The purpose of the study was to characterize impacts of ICS and accompanying antibiotic treatment on the gut microbiome of young turkeys.

Methods: Cecal samples were taken from four flocks over five weeks. Bacterial community DNA was extracted and purified. The 16S rRNA genes were amplified and sequenced using an Illumina MiSeq. The data were processed in QIIME and statistical analysis performed in R.

Results: The turkey cecal microbiome tended to increase in diversity with age and primarily included members of the phyla Firmicutes, Bacteroidetes, and Proteobacteria. Samples fell into two distinct paths, one more diverse than the other. Samples tended to cluster by age instead of flock. Flocks that developed ICS exhibited an overabundance of *Lactobacillus* spp. and *Campylobacter* spp., while other microbiome were more diverse with higher levels of Clostridiales. Antibiotics had no significant impact on the microbiome.

Significance: Patterns of diversity and variability among turkey cecal microbiomes are not well understood at either the individual or the flock scale. This study provided detailed information for individual birds from four flocks. A large number of birds with less diverse microbiomes or low relative prevalence of Clostridiales could serve as indicators for flock susceptibility to ICS, potentially leading to novel strategies to decrease ICS in young flocks.

Poster

P2-140 NGS Based Method for *Enterobacteriaceae* Discrimination and Reliability for *Cronobacter* spp. Identification

Sofia Nogueira[1], Fan Mingzhen[2], David Tomas Fornes[3] and **SANDRA CHAVES**[1]
[1]SGS Molecular, Lisboa, Portugal, [2]Nestec Ltd, Nestlé Research Center, Lausanne, Switzerland, [3]Nestlé, Lausanne, Switzerland

Introduction: **:** Enterobacteriaceae is a large bacterial family, including both harmless and pathogenic genera. *Cronobacter* spp. is a food-born pathogen associated with severe infections in premature infants. Over 90% of *Cronobacter* spp. infections in neonates are related to powdered infant formula (PIF). Presently, most sequencing methods used for bacteria identification are based on 16S rRNA gene which do not allow distinguishing among different genera within Enterobacteriaceae family. SGS Molecular developed a new method based on amplicon sequencing that allows *Enterobacteriaceae* differentiation in complex samples.

Purpose: Apply the *Enterobacteriacea*e identification method for distinguishing *Enterobacteriaceae* strains, including *Cronobacter* spp. and close related non-*Cronobacter* genera in mixed samples.

Methods: A fusion-primer based method was developed, internally validated (validation was performed with 20 Enterobacteriaceae species and 19 mixtures) and adapted by SGS Molecular to become a beta version library preparation kit. The *Cronobacter* spp. study performed at Nestlé Research Center included 15 *Cronobacter* spp. isolated strains and 10 different mixtures of *Cronobacter* and non-*Cronobacter* spp. strains analised by triplicate. After preparation, libraries were sequenced using Ion torrent platform. Sequencing results were analyzed with a dedicated software developed by SGS Molecular.

Results: All the Enterobacteriaceae species tested during the method's validation could be correctly identified. Considering *Cronobacter* spp. evaluation, all isolated strains were properly identified and in all mixtures *Cronobacter* spp. could be distinguished from non-*Cronobacter* spp. strains. With some few exceptions, the Enterobacteriaceae strains analyzed together with *Cronobacter* spp. were properly identified.

Significance: Although some improvements must be performed to this workflow, results obtained suggest that SGS Molecular's kit for Enterobacteriaceae identification allows the distinction of species within this bacterial group, as well as the differentiation of *Cronobacter* spp. from non-*Cronobacter* spp. strains in mixed populations

P2-141 Comparative Genomics of Diarrheagenic *Bacillus cereus* Isolates from Dried Food and Animal Feed

LAURENDA CARTER[1], Hannah Chase[1], Cynthia Stine[2], Charles Gieseker[2], Nicholas Hasbrouck[2], Ashraf Khan[3], Kyuyoung Han[1], Ben Tall[1] and Gopal Gopinath[1]
[1]U.S. Food and Drug Administration, Laurel, MD, [2]CVM, Laurel, MD, [3]U.S. Food and Drug Administration/NCTR, Jefferson, AR

Introduction: The *Bacillus cereus* (Bc) phylogenetic group is thought to contaminate nearly all agricultural products and its members exhibit a wide range of genomic diversity. Bc is responsible for two types of foodborne illness, diarrheal and emetic (vomiting) syndromes.

Purpose: This study describes a strategy that was developed to characterize diarrheagenic Bc strains obtained from food and feed, as well as to evaluate the reliability of using whole genome sequencing (WGS) and a 25 allele MLSA analysis as a viable bioinformatics tool to compare phylogenetic relatedness among strains.

Methods: Bc strains were recovered from dried foods and animal feed. Toxin profiles were determined by a multiplex end point PCR assay using primers derived from the hemolysin BL (*hbl*), nonhemolytic enterotoxin (*nhe*), cytotoxin K (*cytK*), and enterotoxin FM (*entFM*) genes. The genomic diversity of the Bc strains was characterized using MLST analysis of genomes from NCBI and by WGS using MiSeq Nextera XT chemistry.

Results: A single local database consisting of genomes from 36 FDA isolates, 13 Dairy, and 21 Genome groups from NCBI were used in the analysis. A comprehensive 25-gene MLSA strategy was designed to capture the genomic diversity of these isolates. Our analysis, using 25 housekeeping genes, resulted in the identification of alleles in 2,179 positions within the 70 genomes. Phylogenetic analysis showed that only 11 of the FDA genomes clustered with 16 genomes of the NCBI Genome group; the other 25 FDA genomes clustered together in a second group, suggesting a greater intra-species genomic diversity, not previously recognized.

Significance: A comprehensive strategy utilizing bioinformatics showed that the current approach of using MLSA with 25 housekeeping genes and WGS was sufficient to capture the intra-species genomic diversity of Bc isolates. This study establishes an effective protocol for further genomics research of the phylogenetically diverse Bc group.

P2-142 Extended-Spectrum Beta-Lactamase-producing *Escherichia coli* from Meconium of Newborn Calves

LIN TENG, Peixin Fan, Amber Ginn, Joseph Driver and KwangCheol Casey Jeong
University of Florida, Gainesville, FL

Introduction: Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*has become a great concern to public health primarily because of its resistance to third-generation cephalosporins, which are widely used in human healthcare facilities to treat bacterial infections. Although it is controversial, it is commonly believed that food animals acquire antimicrobial resistant (AMR) bacteria by receiving antibiotic treatments.

Purpose: The purpose of this study was to identify the earliest time when animals are exposed to ESBL-producing *E. coli*.

Methods: Meconium samples were collected from the rectal anal junction of 322 newborn calves. ESBL-producing *E. coli* were identified from the samples by plating on MacConkey agar supplemented with Cefotaxime (4 µg/mL). Isolates were further characterized with ChromAgar *E. coli* and CTX-M gene amplification using PCR. Illumina MiSeq was employed for Whole Genome Sequencing (WGS) of 37 strains from 24 calves. After assembly using SPA*des*2.0, nineteen representative strains were selected, based on their Sequencing Types (STs) and whole genome architecture, for further bioinformatics analyses and antimicrobial susceptibility test.

Results: ESBL-producing *E. coli* was detected in 7.5% (24/322) of meconium samples of newborn calves. Following WGS, phylogenetic analysis revealed that these strains clustered into 8 groups that coincided with their STs. All the isolates carried a variety of virulence genes and were resistant to multiple antibiotics, suggesting that these strains may threaten public health if they contaminate food products. In particular, we identified hyper-virulent strains of ST117 that harbored Shiga toxin-encoding genes (*stxAB*), which may cause severe human diseases.

Significance: This was the first study that accessed the prevalence and characterization of ESBL-producing *E. coli* in meconium of newborn calves, indicating animals are even start to be exposed to AMR bacteria in the uterus.

P2-143 Roles of *Staphylococcus Aureus* in Intestine

HEEYOUNG LEE and Yohan Yoon
Sookmyung Women's University, Seoul, South Korea

❖ ***Developing Scientist Competitor***

Introduction: *Staphylococcus aureus* are generally well-known as intoxication foodborne pathogens, and many people annually have suffered from the foodborne illness. Although the pathogen is also isolated from human intestine, and *S. aureus* cells can be ingested with food. The roles of *S. aureus* in intestine have not been studied yet.

Purpose: The objective of this study was to evaluate the roles of *S. aureus* in intestine by *in vivo* study.

Methods: Five weeks old of C57bl/6 mice (*n*=6/group) were purchased from Orientbio in Korea. After 1 week acclimation, 100 µL of *S. aureus* (10^8 CFU/mL) were orally injected to the mice for every 2 days during 2 weeks, and the sham group were injected with same amount of phosphate buffer solution. After 1 week, the mice were scarified, and microbiological analysis, anatomical analysis, immunological analysis, and histopathological analysis were conducted.

Results: *S. aureus* colonies were isolated from sham and *S. aureus*-injected group at 3 and 6 log CFU/g, respectively. In anatomical analysis, mice weight were not significantly different, but the length of small intestine and colon in *S. aureus*-injected group were significantly shorter (P<0.05) than those of sham group. Nitric oxide concentrations in blood samples were slightly lower in *S. aureus*-injected group. In histopathological analysis, there were no difference in histological aspects in small intestine and large intestine for shame and *S. aureus*-injected group.

Significance: These results indicates that orally ingested *S. aureus* can exist in intestine, and potentially promotes microenvironmental conditions that are conducive to the development of inflammatory disease.

P2-144 Patterns of Source Distribution for *Salmonella enterica* Serotype Typhimurium Revealed by Large-scale Whole Genome Sequencing

SHAOKANG ZHANG[1], Dave Boxrud[2], Angie Taylor[2], Chandler Roe[3], Elizabeth Driebe[3], David Engelthaler[3], Paul Keim[3], Eija Trees[4], Efrain Ribot[4], Patricia Fields[4] and Xiangyu Deng[1]
[1]University of Georgia, Center for Food Safety, Griffin, GA, [2]Minnesota Department of Public Health, Saint Paul, MN, [3]Translational Genomics Research Institute, Phoenix, AZ, [4]Enteric Disease Laboratory Branch, Centers for Disease Control and Prevention, Atlanta, GA

❖ ***Developing Scientist Competitor***

Introduction: Routine application of whole genome sequencing (WGS) in the monitoring and surveillance of foodborne pathogens has created a wealth of publicly available genomes and associated metadata, particularly for prevalent pathogens such as *Salmonella enterica* serotype Typhimurium (ST). It is now possible to study population structures of such pathogens from various sources, at unprecedented scales and in a dynamic spatiotemporal context with constant feeds of new data.

Purpose: The goals of this work were to (1) build a comprehensive phylogeny of ST; (2) identify phylogenetic lineages associated with particular sources; and (3) characterize identified groups/lineages with the purpose of exploring WGS as a method for ST source tracking.

Methods: Genomes of 1,268 ST isolates, collected between 1930 and 2014, were studied. The collection included clinical isolates (*n*=107) representing diversity of ST molecular subtypes (PFGE and MLVA) from CDC and public genomes of various sources and geographic locations (46 US states and 39 countries; *n*=1,161). A comprehensive phylogeny was constructed using core-genome SNPs. Gene contents and pseudo genes were compared among major population groups. Overall metabolic potentials of selected strains from major lineages were evaluated with Biolog Phenotype Microarrays. Association between specific lineages and particular sources (e.g., poultry, swine, bovine, avian etc.) was statistically evaluated.

Results: Ten major population groups were identified. Clustering of isolates from the same source was observed in multiple cases, including clades overrepresented by isolates from poultry, bovine, swine, and avian samples. Evolutionary analyses suggest that a least two of these clades originated in recent decades and appeared to be associated with meat production. Representative isolates from an avian clade and a swine clade displayed distinct metabolic profiles from others featuring systemic incapability in utilizing multiple substrates.

Significance: This study built a comprehensive population structure of ST, which provides a framework for evaluating and exploring WGS for foodborne pathogen source tracking.

P2-145 Transcriptomics is a Useful Approach for Investigating the Effects of Long-term Storage on *Salmonella enterica* Serotype Montevideo Survival When Spiked on Oregano

LAURA EWING[1], Gopal Gopinath[1], Nicole Addy[1], Darcy Hanes[2] and Junia Jean-Gilles Beaubrun[1]
[1]U.S. Food and Drug Administration, Laurel, MD, [2]U.S. Food and Drug Administration, CFSAN, Laurel, MD

Introduction: Understanding pathogen survival in low-moisture foods, such as spices, can yield useful information for mitigating outbreaks and improving detection methods. *Salmonella* is especially persistent in surviving over long periods of time in stressful environments, as is evidenced by recent outbreaks associated with spices. RNA-seq is a relatively new, high-throughput technology used for comprehensive transcriptomic analysis.

Purpose: This study investigates whether stressing *Salmonella* via long-term storage and inoculating on oregano results in detectable gene expression changes and if observed changes suggest potential adaptive processes that enable pathogen survival on spices.

Methods: *Salmonella enterica* serotype Montevideo was inoculated in 250mL of TSB, incubated 16-24 hr at 37°C, and subsequently transferred to 4°C for long-term storage. Monthly, 500 ul of the inoculum stored at 4°C was spiked onto 25g oregano with 250 mL TSB plus 2% v/v corn oil, and incubated 16-24 hr at 37°C with shaking. Total RNA was extracted, enriched for mRNA, sequencing library generated and sequenced on the Illumina Miseq platform. GenBank CDS annotations were processed in CLC Bio Workbench.

Results: Of 4367 loci, 61% displaying discernable number of sequence reads were taken for further analysis. Differential transcription was observed over time. Genes involved in a broad spectrum of processes from housekeeping to stress response were observed. They include chaperone, cold shock, SOS-response, DNA replication and repair proteins, proteases, methylase, and those involved in ammonia reduction and alternative metabolic pathways.

Significance: Transcriptomics is a powerful tool for capturing snapshots of molecular changes in gene expression over time. These molecular changes most likely reflect adaptive physiological changes that enhance the survival of *Salmonella* in highly stressful environments, like low-moisture foods such as spices. This information can be used to advance the FDA's mitigation, detection, and eradication of foodborne pathogens in the food supply.

Poster

P2-146 Comparative Genomics of *Bla$_{ctx-M-65}$*-resistant Clinical Strains of *Salmonella enterica* serovar Infantis from Peru and Resistant Strains from Chicken, Cattle and Humans

Gopal Gopinath[1], Hannah Chase[1], Jayanthi Gangiredla[1], Isha Patel[1], Nicole Addy[1], Junia Jean-Gilles Beaubrun[1], Baoguang Li[1], Christopher Elkins[1], Flavia Negrete[1], Samantha Finkelstein[1], HyeJin Jeong[1], ChaeYoon Lee[1], Kyuyoung Han[1], Shaohua Zhao[1], Gregory Tyson[1], Heather Tate[1], Drake Tilley[2], Mark Simons[2], Andrea McCoy[2], Rina Meza[2], Allison Brown[3], Cindy Friedman[3] and **BEN TALL**[1]

[1]U.S. Food and Drug Administration, Laurel, MD, [2]U.S. Naval Medical Research Unit No. 6, Callao, Peru, [3]Centers for Disease Control and Prevention, Atlanta, GA

Introduction: Extended-spectrum β-lactamase (ESBL) genes are capable of hydrolyzing commonly used β-lactam antibiotics, including third-generation cephalosporins reserved for treatment of severe infections.

Purpose: A detailed genomic comparison of Peruvian clinical *Salmonella enterica* serovar Infantis strains bearing a *bla*$_{CTX-M\ 65}$ (cefotaximase) gene in relation to similar strains from environmental and food sources is presented.

Methods: Three hundred twenty-two clinical *S*. Infantis strains were obtained from patients with diarrhea from 1997-2014, representing a network of 13 Peruvian hospitals. These strains were characterized using PCR; microarray; whole genome sequencing (WGS); whole genome core gene (wgCGA) with 2700+ *Salmonella* core gene loci; kSNP3; in-house R; and ResFinder analyses for *bla*$_{CTX-M-65}$ genes. Serotype was confirmed with SeqSero, Premitest, and microarray.

Results: Results showed that 206 (64%) of the strains associated with 6 different hospitals were PCR-positive for *bla*$_{CTX-M-65}$ which was confirmed by WGS in 26 strains. Other antibiotic resistance genes: *bla*$_{CTX-M-\ 15}$; *bla*$_{SHV-129}$; *bla*$_{OXA-2;}$ *bla*$_{TEM-1B}$; and *bla*$_{CTX-M-2}$ were also noteworthy. Genomic reconstruction revealed a highly conserved, previously reported 320 kb plasmid in all *bla*$_{CTX-M-65}$-positive strains. For comparison, other *S.* Infantis genomes (100+) of environmental, animal, and human origin were obtained from NCBI; intra-serovar differences were seen with these strains compared to Peruvian clinical strains. Microarray, wgCGA and kSNP3 separated the *S.* Infantis strains into distinct clusters. Microarray analysis, which included the mobilome region, provided a higher resolution of *bla*$_{CTX-M-65}$ *S.* Infantis strains. Genomic characterization showed that the *bla*$_{CTX-M\ 65}$ genes among these Peruvian clinical *S.* Infantis strains were comparable to resistant strains from environmental and animal origin.

Significance: High similarity between *bla*$_{CTX-M-65}$ positive *S.* Infantis strains from hospitals, and other sources, is a matter of concern for public health. The results of the study demonstrate a need to expand genomic targets to detect *bla*$_{CTX-M-65}$-resistant strains including those associated with the food supply.

P2-147 Transcriptome Analysis of *Salmonella enterica* Newport i*n Planta* after Desiccation and Postharvest Sanitization

LAUREL DUNN[1], Dara Smith[1], Dean Kopsell[2] and Faith Critzer[1]

[1]University of Tennessee, Department of Food Science, Knoxville, TN, [2]University of Tennessee, Department of Plant Sciences, Knoxville, TN

❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* is known to survive on and colonize produce, and is particularly adept at colonizing tomato (*Solanum lycopersicum*) plants. Post-harvest sanitizers are implemented to limit cross-contamination during postharvest washing, but do little to remove bacteria from contaminated produce. Little is known about genetic mechanisms utilized by *Salmonella* to survive in these environments.

Purpose: Determine genes required for survival of *Salmonella* on tomatoes during simulated environmental conditions and postharvest washing.

Methods: Dwarf tomato plants were placed in an environmental chamber with *Salmonella* Newport inoculated fruit for 2 h (29.4°C, 60% RH) or for a 24 h cycle mimicking average July field conditions in east TN (day- 29.4°C, 60% RH: morning/evening- 23.9°C, 75% RH: night-20°C, 89% RH). Tomatoes were then harvested and washed in water, 50 ppm chlorine, 40 ppm peroxyacetic acid (PAA), or a no rinse (NR) control for 1 min to simulate postharvest washing. *Salmonella* RNA was stabilized, extracted, sequenced and relative expression of the transcriptome was compared.

Results: When compared to the 2 h NR control, 24 h NR *Salmonella* upregulated genes required for TCA cycle II 4-fold and aerobic respiration 3.4-fold. Glycolytic pathway associated genes displayed a range of response from up to 36.9 fold for 24 h water; 2 h water treatments only had downregulation (up to 5-fold) of glycolytic pathways. Twenty-four hour chlorine downregulated UMP biosynthesis 65.9-fold, which was not evident after only 2 h on tomato fruit. Arginine and aminobutyrate degradation pathways were slightly upregulated (3.2-fold), while arginine biosynthesis pathways were downregulated up to 67.3-fold for 24 h PAA treatment. Two hour PAA treatment resulted in 16.4-fold upregulation of autotrophic ammonia oxidation and an 8-fold downregulation of aerobic ammonia oxidation.

Significance: Understanding the mechanisms of *Salmonella* survival on plants will provide insight which can lead to better food safety interventions for the produce industry.

Poster

P2-148 *Salmonella* Newport Gene Expression Profile on Sterile Tomato Seedlings is Indicative of Mitigating Plant Stress

ANGELA FERELLI[1], Samantha Bolten[2] and Shirley Micallef[1]

[1]University of Maryland, College Park, MD, [2]U.S. Department of Agriculture–ARS, Beltsville, MD

Introduction: Plants recognize pathogens via pathogen-associated molecular patterns (PAMPs), mounting basal defense responses that are modulated by reactive oxygen species (ROS) and reactive nitrogen species (RNS). Plants are capable of recognizing *Salmonella* PAMPs, however the resulting bacterial responses are understudied.

Purpose: A series of *Salmonella enterica* genes were investigated in *S*. Newport associated with tomato seedling leaves.

Methods: To evaluate the *Salmonella* response in the tomato phyllosphere, sterile three leaved-seedlings of tomato cv. Heinz were challenged with five log CFU *S*. Newport/mL, delivered as 10×1 µL spots on leaf surfaces. Samples were collected six hours post-inoculation and cells immediately fixed in RNAProtect Bacteria. Total RNA was extracted and cDNA synthesized for amplification with primers targeting 14 genes needed for ROS mitigation *(soxR, marA),* nitric oxide detoxification (*yeaR, ygbA, yoaG, yjbE*), antimicrobial stress tolerance (marA, wza), tryptophan metabolism (*trpD, trpE*), carbon metabolism (*nmpC*, *lamB*), anaerobic conditions (ttrA, hcp) and *STM1808,* on an ABI Step-One Plus using SYBR as a reporter. Relative expression was compared to cells grown on Tryptic Soy Agar. Data was analyzed using the ddCt method with *rpoD* as an endogenous control.

Results: *Salmonella* Newport, epiphytic on tomato leaves, presented a significantly different gene expression profile than *S.* Newport on nutrient agar. A priori comparisons report genes that are important for antibiotic resistance, biofilm formation, and NO stress. The genes *marA, trpD, trpE, wza, yeaR, ygbA, hcp* and *yjbE* were expressed at significantly higher levels on tomato compared to nutrient agar, with a greater than eight-fold increase (P<0.05). Significant down-regulation of genes associated with carbon metabolism *nmpC* and *lamB* was also observed with a greater than or equal to two-fold reduction (P<0.05).

Significance: Understanding how enteric pathogens and plants mutually respond is crucial to fully describing these interactions; the knowledge of which can aid in augmenting pre-harvest risk analysis models and Good Agricultural Practices.

P2-149 Transcriptome Response of *Salmonella* Newport to Oxidative Antimicrobials

DARA SMITH, Laurel Dunn and Faith Critzer
University of Tennessee, Department of Food Science, Knoxville, TN

❖ ***Developing Scientist Competitor***

Introduction: Transfer of foodborne pathogens such as *Salmonella* from agricultural waters to produce presents a prominent issue in food safety. While mitigation strategies exist to reduce or eliminate this possibility, many factors such as environmental conditions and microbial response can affect the overall efficacy of these methods. Investigating genetic response to commonly used oxidative antimicrobials such as sodium hypochlorite (NaOCl) and peroxyacetic acid (PAA) may elucidate aspects of this complex interaction. Further understanding of pathogen response to antimicrobials may increase efficacy of disinfection systems.

Purpose: The purpose of this study was to analyze the genetic response of an outbreak strain of *Salmonella* Newport upon exposure to two commonly used antimicrobials.

Methods: *Salmonella* Newport was exposed to NaOCl (pH 6.5) and PAA at concentrations of 0, 10 and 20 ppm each for 30 seconds. Treatments were conducted with two samples per treatment repeated in duplicate. Following exposure, cells were isolated and RNA extracted for sequencing. Transcriptomes were compared between treatments and control (0 ppm) for significantly up or down-regulated genes (FDR $P \leq 0.05$).

Results: Significant upregulation of electron transport chain-associated genes was noted in both PAA treatments up to 47.4-fold and 54.0-fold, respectively ($P<0.05$), and 20 ppm PAA showed up to a 296.0 and 195.2-fold increase in citrate and shikimate degradation, respectively. While 10ppm NaOCl treatments resulted in minimal significant induced changes comparatively, upregulations were noted in cellular membrane associated proteins (up to 3.5-fold), protein synthesis associated components (up to 21.4-fold), as well as redox-stress associated transcriptional regulation (up to 2.4-fold). Upregulation of protein synthesis components was also seen in 20 ppm NaOCl up to 242.2-fold and phage-associated genes up to 40.3-fold.

Significance: Observed trends in gene induction lend to the need for further investigation of microbial response to antimicrobials to ensure effective mitigation strategies.

P2-150 Next Generation 16S rRNA Microbiome Analyses of a Mixed Culture MPN from Chicken Breast Samples Inoculated with a *Salmonella*

SUN AE KIM, Si Hong Park, Sang In Lee and Steven Ricke
University of Arkansas, Fayetteville, AR

Introduction: Our previous study developed a novel *Salmonella* Typhimurium quantification method using a most-probable-number (MPN) combined with qPCR and a shortened incubation time (MPN-qPCR-SIT). In this detection procedure, growth of non-target background microbiota during incubation time can be a major factor in the accuracy of enumeration.

Purpose: The aim of this study was to determine bacterial compositional shift occurring over the incubation time period using microbiome analysis from chicken breast samples inoculated with mixed cultures (*S.* Typhimurium with other bacteria from chicken rinsates) at various incubation times.

Methods: Chicken breast samples inoculated with *S.* Typhimurium in mixed culture were incubated at 0, 4 (reflective of the incubation times needed for MPN-qPCR-SIT), and 24 h (the incubation times needed for conventional plating, conventional MPN, and qPCR) and incubated samples were subjected to microbiome analysis.

Results: The R value from PCoA plot was 0.56 and it implied that there was significant dissimilarity among different incubation time groups in terms of phylogenetic distance. Bacterial composition of samples shifted over the incubation time period. At a phylum level, the abundance of Firmicutes and Actinobacteria significantly increased during incubation while the relative abundance of Proteobacteria decreased over the incubation time period. At a genus level, the most significant shift occurred with *Enterobacteriaceae* and their levels of abundance were increased from 17.99% at 0 h to 53.50 and 90.69% at 4 and 24 h incubation samples, respectively. *Salmonella* abundance was only 0.03% at 0 h sample but increased with the corresponding incubation times that included 4 h (0.08%) and 24 h (0.18%). The sequence identified *Enterobacteriaceae* appears to be reflecting the increases in artificially inoculated *Salmonella*.

Significance: These results indicated that enrichment procedure for a shortened time used in the MPN-qPCR-SIT can sufficiently support growth of target bacteria to ensure reliable detection and quantification. Also, *Enterobacteriaceae* could be used as effective indicator organisms for potential presence of *Salmonella* in microbiome analyses.

P2-151 Comparative Genomics Confirms Persistence of *Salmonella* Serovar Newport in Environmental Waters of Southern and Central Georgia

Christopher Grim[1], Meimin Wang[1], Susan Leonard[1], Erin Lipp[2], John Maurer[3], Michele Ray-Russell[4], George Vellidis[5], Mark Mammel[1], Christopher Elkins[1] and **BAOGUANG LI**[1]
[1]U.S. Food and Drug Administration–CFSAN, Laurel, MD, [2]University of Georgia, Athens, GA, [3]University of Georgia, Athers, GA, [4]University of California-Davis, Davis, CA, [5]University of Georgia, Tifton, GA

Introduction: There has been and continues to be a significant investment in environmental surveillance activities to detect human health hazards, such as presence, frequency and abundance of bacterial pathogens. Traditionally, these types of studies are conducted and reported by a single research group. Whole genome sequencing (WGS) has gained wide usage as a tool for epidemiological and evolutionary investigations.

Purpose: The purpose of this study was to compare and determine relatedness among isolates of *Salmonella* serovar Newport from three distinct surveillance activities, which all took place in a discrete geographical area, namely the rivers and surface waters of Southern and Central Georgia, USA in agricultural areas.

Methods: Forty-one isolates of *S.* Newport were compared by reference-based whole genome SNP analysis, average nucleotide identity, and tetranucleotide frequency to determine relatedness. Representative strains from each identified cluster were compared by reciprocal BLASTP analysis to determine unique and clade specific genomic features.

Results: WGS-based SNP analysis separated the isolates into two major clades, one with 16 members (Lineage II) and the other with 25 (Lineage III). All the Lineage II isolates came from a single study and single source. They all harbor a large plasmid of approximately 142 kb, which is highly homologous with the antibiotic resistance plasmid, pSN254, from Newport strain SL254. The Lineage III clade was further divided into three subclades, with

two of them comprising isolates from more than one study. Comparative genomics corroborated these results with sub-clade members possessing group-specific, as well as inter-group specific, prophage and other mobilome elements.

Significance: These results suggest that certain strains of *S*. Newport can persist in defined geographic areas over time.

P2-152 Transcriptional Profiling of *Salmonella* Montevideo Exposed to the Probiotic *Lactobacillus animalis* NP51

DIANA AYALA, Mindy Brashears and Kendra Nightingale
Texas Tech University, Lubbock, TX

Introduction: Non-typhoid *Salmonella* is one of the most important causes of foodborne illness worldwide. In the United States, between 800,000 and 1.4 million cases of *Salmonella* infections occur annually; a common source of salmonellosis is direct contact with animals carrying the pathogen. *Salmonella* Montevideo has been commonly isolated from feedlot cattle, and it is among the top ten serotypes that cause human disease. *Lactobacillus animalis* NP51 is a direct-fed microbial (DFM) used as a pre-harvest intervention to reduce *Salmonella* colonization of the bovine gastrointestinal tract and subsequent fecal shedding that contributes to carcass contamination by this foodborne pathogen.

Purpose: This study used RNA-Seq to elucidate the gene expression profile of *Salmonella* Montevideo grown in the presence of *Lactobacillus animalis* NP51.

Methods: Bacterial strains were grown overnight at 37°C in media shown to support co-culture of both *Salmonella spp.* and *Lactobacillus* spp. Overnight cultures were diluted into fresh media contained in dialysis tubes. Tubes were, then, placed in falcon tubes containing media with or without NP51 (control). Samples were incubated at 37°C until mid-logarithmic phase was reached. Total RNA was extracted from three biological replicates; treatment and control samples were rRNA depleted, followed by bar-coding of individual samples. RNA-Seq libraries were prepared and sequenced on a MiSeq instrument. Raw data sets were assembled de novo; DNAStar Array Star was used to analyze gene expression profiles, and Blast2go software was used to annotate differentially expressed genes.

Results: A total of 339 genes were found to be differentially expressed at two-fold change and 95% confidence intervals; 50.1% (*n*=170) genes showed reduced expression, while 49.85% (*n*=169) increased their expression. Transporter activity and binding were among the molecular functions up-regulated; motility and virulence-associated genes were found to be down-regulated.

Significance: This study provided important insights into probiotic-pathogen interactions and mechanisms by which *Lactobacillus animalis* NP51 prevented *Salmonella* Montevideo from colonizing the host.

P2-153 Identification of Putative Surface Proteins Involved in Adherence of *Listeria monocytogenes* on Abiotic Surfaces

Peter Muriana and **HUNG KING TIONG**
Oklahoma State University, Stillwater, OK

❖ ***Developing Scientist Competitor***

Introduction: Attachment of bacterial cells to abiotic surfaces is a prerequisite for biofilm formation and contamination of food products. Some strains of *Listeria monocytogenes* adhere more strongly than others and more likely contribute to contamination found in food processing facilities.

Purpose: Our purpose was to identify potential surface adhesins in *L. monocytogenes*.

Methods: A surface protein extraction method was optimized for investigative comparison of the surface sub-proteomes and sub-transcriptomes of surface-adherent variants of *L. monocytogenes* cells (planktonic vs. sessile) using Orbitrap LC-MS/MS and RT-qPCR, respectively. Significant protein and mRNA differential expressions were determined for multiple biological (2, 3) and technical (3, 2) tests using Fisher's exact ($P<0.02$) and Student's t-tests ($P<0.05$), respectively.

Results: LC-MS/MS comparative analyses for five surface extraction methods revealed that the UB-Ghost extraction method, whereby cells were pre-bled for cytoplasmic components, produced higher purity extracts (i.e. fewer number of cytoplasmic unique peptides) (153) than LiCl (190), UB (219), trypsin-BICAM-sucrose (211), and trypsin-Tris-sucrose (231), suggesting that this extraction method be used for subsequent investigations. LC-MS/MS analyses and spectral counts for surface sub-proteomes of the planktonic cells of adherence-variant strains of *L. monocytogenes* identified differentially expressed proteins which were primarily detected in strongly-adherent *L. monocytogenes* cells (≥5-fold, 43 proteins), some of which were novel moonlighting proteins. RT-qPCR gene expression analyses of 15 select surface proteins of among attached and planktonic cells of *L. monocytogenes*,prepared at 30°C and 42°C, revealed 7 over-expressed genes (between 2- and 102-fold expression), encoding unknown (2), virulence (3), and non-virulence (2) functions, either in sessile cells alone (3) or in both planktonic/42°C and sessile cells (4).

Significance: Food outbreaks associated with bacterial adherence may involve novel surface adhesins. Our findings suggest a group of additional surface adhesins that may contribute to a greater understanding of molecular mechanisms involved with adherence in *L. monocytogenes* to abiotic surfaces.

P2-154 Whole Genome Sequencing of *Listeria monocytogenes* Strains Carrying Loss of Function Mutations in *inlA* Supports These Strains are Evolving Away from a Pathogenic Lifestyle

PETER COOK[1], Henk Den Bakker[1], Guy Loneragan[2] and Kendra Nightingale[1]
[1]Texas Tech University, Lubbock, TX, [2]Texas Tech University, Department of Animal and Food Sciences, Lubbock, TX

❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* is capable of thriving as a saprotroph and causing invasive disease as an intracellular pathogen. Bacterial adaptation to a lifestyle can occur quickly by loss-of-function (LOF) mutations that increase fitness in a given niche. Previous studies support that *L. monocytogenes* strains carrying LOF mutations in *inlA* have adapted to thrive in non-host environments as a tradeoff for loss of full virulence. If these strains are indeed adapted to non-host environments, we assume additional genes involved in host-pathogen interactions carry similar LOF mutations.

Purpose: This study aimed to use Whole Genome Sequencing (WGS) to characterize *L. monocytogenes* strains with naturally occurring premature stop codons (PMSCs) in *inlA* and identify genome-wide mutations that may contribute to evolution towards a non-pathogenic lifestyle.

Methods: 43 *L. monocytogenes* isolates carrying naturally occurring PMSCs in *inlA* were characterized by WGS. The resulting genome sequences were analyzed by constructing a phylogenetic tree followed by comparing each generated sequence to the closest available clonal complex (CC) sequence without a PMSC in *inlA*. Variant nucleotides were annotated to determine if each mutation resulted in a potential LOF.

Results: The 43 strains fell into eight previously identified CCs. Analyses of each CC independently resulted in the identification of several LOF mutations across the CC level, but not at the lineage level. LOF mutations potentially associated with decreased fitness in a host were identified in genes for sugar hydrolase enzymes (*lmo2733*), genes for the production of menaquinone (*lmo1201* and *lmo1932*), and genes for DNA repair and replication (*mutS* and *dnaG*).

Significance: The LOF mutations identified in this study suggest that *L. monocytogenes* carrying a PMSC in *inlA* are independently losing genes necessary for survival and replication intestinally and intracellularly. *L. monocytogenes* carrying a PMSC mutation in *inlA* are likely accumulating mutations that result in loss of the ability to lead a pathogenic lifestyle.

P2-155 Determining if Phylogenetic Relatedness of *Listeria monocytogenes* Isolates Corresponds to Persistence in Poultry Processing Plants Using Whole-Genome Sequencing

LAUREN HUDSON[1], Mark Berrang[2], Richard Meinersmann[2], Xiangyu Deng[3] and Mark Harrison[1]

[1]University of Georgia, Athens, GA, [2]U.S. Department of Agriculture-ARS-USNPRC, Athens, GA, [3]University of Georgia, Center for Food Safety, Griffin, GA

❖ ***Developing Scientist Competitor***

Introduction: Controlling *Listeria monocytogenes* on ready-to-eat meat and poultry products and in food processing facilities is challenging. Surveys have found that some *L. monocytogenes* types are more persistent in processing facilities than others, but the reason is unknown. It is possible persistent strains vary genetically from transient ones, giving them an advantage that aids in attachment to surfaces and biofilm formation in processing facilities.

Purpose: The goal of the study was to use whole genome sequencing (WGS) to determine phylogenetic relationships among *L. monocytogenes* strains and to determine if these relationships correspond to persistence.

Methods: In two previous longitudinal studies of *L. monocytogenes* in poultry processing plants, isolates were collected, separated into discreet subtypes by sequencing of *act*A gene, and identified as either transient or persistent. In the present study, 170 genomes were extracted, sequenced on the Illumina MiSeq platform, *de novo* assembled, and annotated. Genomes with acceptable assembly quality scores (160 isolates total) were analyzed to determine phylogenetic relatedness. Repeat sequences, phage sequences, and recombination regions were trimmed out. Core-genome alignment, SNP calling, and phylogenetic tree construction were performed using Harvest suite including Parsnp.

Results: Clades present on the constructed phylogenetic tree follow closely with subtypes determined via sequencing of *act*A gene in the original studies. No clear phylogenic relationship trends between persistent and transient subtypes analyzed could be determined. Raw sequence data and contigs will be added to the appropriate publically accessible GenBank database to be available for further analysis.

Significance: Additional analysis is needed to identify genetic factors that may be involved or interact with regulation of genes that influence ability of some strains to colonize and survive on food and non-food contact surfaces, while others are less likely to do so. In addition, this data shows WGS, along with phylogenetic analysis, is a viable option for subtyping *L. monocytogenes* isolates.

P2-156 A Comprehensive Evaluation of the Genetic Relatedness of *Listeria monocytogenes* Serotype 4b Variant Strains

LAUREL BURALL[1], Christopher Grim[2], Mark Mammel[1] and Atin Datta[3]

[1]U.S. Food and Drug Administration–CFSAN, Laurel, MD, [2]U.S. Food and Drug Administration, CFSAN, Laurel, MD, [3]U.S. Food and Drug Administration - CFSAN, Laurel, MD

Introduction: Recently, we identified a link between four listeriosis incidents and a variant of *Listeria monocytogenes* serotype 4b, 4bV. These 4bV strains have a 4b genomic backbone with a 6.3kb DNA segment from lineage II. Isolates from these incidents were highly related and three of these incidents involved produce. The increased association of 4bV isolates raised the concern that 4bV were more prevalent in food and clinical samples than previously thought.

Purpose: We evaluated a more comprehensive strain collection to better establish the genomic diversity of 4bV and identify trends that could be related to food contamination and human listeriosis.

Methods: 4bV isolates were identified from the NCBI Nucleotide collection (nr/nt) and SRA databases using BLASTn. Relatedness was evaluated using the CFSAN SNP Pipeline and six gene multi virulence locus sequence typing (MVLST). Phylogenetic results were compared with available metadata.

Results: A total of 367 *L. monocytogenes* 4bV strains were identified from either clinical isolates or BLAST interrogation of 6830 NCBI sequences. Over half of the isolates originated from clinical sources, many from apparent sporadic cases. Strains originated from the USA, Australia, and Chile. The 4bV strains diverged from traditional 4b strains and formed seven clusters when analyzed with the CFSAN SNP Pipeline. When MVLST was used to examine phylogeny, 87% belonged to a single cluster, comprised of strains from the two largest SNP-based 4bV clades. These clades represented geographically distinct regions, atypical foods, and contained historical isolates from the early 2000s.

Significance: The differing phylogenetic results suggest that 4bV strains undergo varied selection processes, restricting diversity across the six virulence loci compared to core genomic variability. These data show that 4bV includes multiple genomic groups and may have adaptations leading to improved survival in foods and/or processing environments. Such knowledge may provide guidance to better control *Listeria* contamination.

P2-157 Prevalence, Distribution, and Comparative Genomics of a Hemolysin III Gene (COG1272) and Related Hemolysin Genes among *Cronobacter* spp.

CHAEYOON LEE, HyeJin Jeong, HaNa Kwon, Kyuyoung Han, SeonJu Choi, SoHyun Kim, Jeong Woo Lee, Jung Youn Do, Samantha Finkelstein, Flavia Negrete, Hannah Chase, Isha Patel, Jayanthi Gangiredla, Gopal Gopinath and Ben Tall

U.S. Food and Drug Administration, Laurel, MD

Introduction: The *Cronobacter* genus is comprised of *C. sakazakii* (Csak), *C. malonaticus* (Cmal), *C. turicensis* (Ctur), *C. universalis* (Cuni), *C. condimenti* (Ccon), *C. muytjensii* (Cmuy) and *C. dublinensis* (Cdub); all of these except for Ccon, can cause foodborne infections. Several studies predict that *Cronobacter* spp. possess a hemolysin III homolog (COG1272) which is thought to play a role in pathogenesis. However, its prevalence and phylogenetic relatedness among *Cronobacter* species are not well studied.

Purpose: In an effort to better understand the prevalence and phylogenetic relatedness of COG1272, 388 *Cronobacter* strains were analyzed by PCR, DNA microarray (MA), and whole genome sequencing (WGS) analyses.

Methods: Strains were identified using species-specific PCR assays targeting *rpoB* and *cgcA* genes. PCR and MA analyses were performed using previously published primers and a pan-genomic DNA microarray. WGS was conducted using a MiSeq platform with Nextera chemistry.

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Results: PCR analysis showed that COG1272 was present in 285/298 (95.6%) Csak strains; 3/7 (42.9%) Cmuy strains; 13/33 (39.4%) Cmal strains; 3/12 (25%) Ctur strains; 4/37 (10.8%) Cdub; and 1/1 Cuni strains, respectively. Ccon was PCR-negative for COG1272. However, MA and WGS showed that Ccon possessed a COG1272 orthologue, but Cuni did not. MA also showed the presence of 27 other hemolysin-related alleles which suggest that the PCR result for Cuni identified a related hemolysin, not COG1272.

Significance: Previously, prevalence of COG1272 was unknown due to the use of less specific identification techniques (i.e., amplification of 16s rDNA). In the current study, we show that COG1272 is present in all *Cronobacter* species except for Cuni. Through the use of MA and WGS, in addition to COG1272, 27 other hemolysin-related genes were found. The current study establishes a powerful platform for further genomics research of this genus, a prerequisite towards development of future countermeasures for this important foodborne pathogen.

P2-158 Characterization of Australian *Escherichia coli* O111 Isolates from Human and Cattle Sources

ROBERT BARLOW[1], Kate McMillan[1], Theo Allnutt[2], P. Scott Chandry[3], Narelle Fegan[3] and Glen Mellor[1]

[1]CSIRO Agriculture & Food, Brisbane, Australia, [2]Deakin University, Geelong, Australia, [3]CSIRO Agriculture & Food, Werribee, Australia

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) O111 rose to prominence in Australia following the Garibaldi brand mettwurst outbreak in 1995. Between 2001 and 2009, STEC O111 were found to be associated with 13.7% of all notified STEC infections. Cattle are a reservoir of STEC, however little is known about the relationship between O111 isolates from cattle and human clinical sources.

Purpose: This study genomically characterized a selection of STEC O111 isolates from cattle and human clinical sources.

Methods: A total of 33 O111 isolates (11 from cattle and 22 humans), collected between 1995 and 2015, were included in the study. Isolates were characterized using next generation sequencing based analysis for multi-locus sequence type (MLST), comparative genome fingerprinting, pangenome content, and single nucleotide polymorphisms (SNPs).

Results: STEC O111 from cattle sources were either O111:H8 ST294 (45%) or O111:H- ST16 (55%). Human isolates were, mainly, O111:H8 of either ST294 (64%) or ST16 (18%) and O111:H11 ST21 (14%). Theta intimin was identified in 30/33 (91%) of all isolates. Carriage of *stx* correlated well with serotype with O111:H8 likely to harbor stx_{1a} and stx_{2a}; whereas O111:H- were typically stx_{1a} only, regardless of source. The three O111:H11 isolates from human clinical sources carried $stx_{1a}$and beta intimin, a profile typical of STEC O26 isolates. Further genetic analysis confirmed that these strains closely resembled typical STEC O26 isolates from Australia.

Significance: Characterization of isolates from cattle and human clinical sources determined that there are strong relationships between O111 isolates from cattle and humans. However, O111 isolates from humans demonstrated a greater tendency to harbor stx_{2a}. This study also reported the first description of a hybrid O111/O26 clone present in human clinical samples. The ability of the *E. coli* genome to undergo recombination creates challenges for those aiming to establish STEC detection protocols with greater discriminatory power.

P2-159 Comparison of Two Diagnostic Methods to Detect Five Different Bacterial Pathogens Associated with Porcine Respiratory Disease Complex (PRDC) and Investigation of Their Prevalence in Pathologic Lung Tissues in Korea

KUN TAEK PARK, Chan Hee Lee, Young Kyung Park, Chung Wung Kim, Sook Shin and Yong Ho Park

Seoul National University, Seoul, South Korea

Introduction: Porcine respiratory disease complex (PRDC) greatly affects the health and production of pigs. PRDC is polymicrobial disease caused by infection of various combinations of primary and secondary bacterial pathogens including *Pasteurella multocida* (PM), *Mycoplasma hyopneumoniae* (MH), *Streptococcus suis* (SS), *Actinobacillus pleuropneumoniae* (APP), and *Haemophilus parasuis* (HP). It is important to investigate the relative prevalence of bacterial pathogens in PRDC and efficient identification methods to control the disease.

Purpose: The current study was performed to compare two diagnostic methods to detect the five causative bacterial pathogens in lung tissues and to investigate the distribution of the bacterial pathogens in Korea.

Methods: A total of 271 lung samples with pathologic lesion were obtained from two slaughterhouses in Korea. Two diagnostic methods were used to detect the bacterial pathogens: i) PCR following DNA extraction from tissues, and ii) Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) following bacterial culture on blood agar.

Results: Among 271 tissue samples, PCR detected all the five pathogens, with the highest detection rate for MH (n = 141, 52%) followed by SS (14, 5.1%), HP (5, 1.8%), APP (5, 1.8%), and PM (4, 1.4%). In contrast, MALDI-TOF following culture method detected only SS (29, 10.7%), PM (29, 10.7%), and APP (1, 0.3%). When the results are combined, the overall detection rates were, from highest to lowest: MH (141, 52%) followed by PM (33, 12.2%), SS (29, 10.7%), APP (6, 2.2%), and HP (5, 1.8%).

Significance: The results indicate that the use of a combinational method of PCR and culture might be a useful way to increase the sensitivity for detection of PRDC pathogens and that the predominant bacterial pathogens for PRDC in Korea are MH and PM. This study provides useful information to control the diseases, which will increase the productivity and animal health on pig farms.

P2-160 Impact of Temperature Dependence of Water Activity on *Salmonella* Inactivation in a Multicomponent Food System

NATHAN ANDERSON[1], Yuqi Luo[2] and Elizabeth Grasso-Kelley[2]

[1]U.S. Food and Drug Administration, Bedford Park, IL, [2]Illinois Institute of Technology, Bedford Park, IL

Introduction: *Salmonella,* which is often inactivated by heat-based processes, has greater thermal resistance at low water activity (a_w). Prior research revealed that a_w did not change appreciably with increasing temperature in a multicomponent food system formulated with 55.9% fat; however, a_w increased in high-protein and high-carbohydrate formulations and decreased in high-fat formulations. Though a_w is temperature dependent and impacted by food composition, the influence of these factors on *Salmonella* inactivation in low a_w multicomponent foods is not well understood and often ignored in process validation.

Purpose: To determine the influence of food composition and temperature dependence of a_w on thermal resistance of *Salmonella* in a multicomponent food system.

Methods: Food matrices, comprised of rice flour, soybean oil and soy protein powder, were formulated to contain high-carbohydrate (67.3%), high-protein (85.8%) and different levels (55.9% and 87.0%) of fat content. Each matrix was inoculated with *Salmonella* Agona then stored under controlled humidity to equilibrate a_w. Samples, ~0.7g, were loaded into aluminum test cells, which were heated at 85°C in a water bath. Samples were cooled, diluted in buffered peptone water, and enumerated on tryptic soy agar supplemented with yeast extract. D-values were calculated and results analyzed by t-test.

Results: Thermal resistance of *Salmonella* was lower in formulations where a_w increased and higher in the formulation where a_w decreased at elevated temperature. D-values for *Salmonella* in the high-carbohydrate and high-protein formulations (3.09±0.11 min and 3.13±0.10 min) were significantly lower (P<0.05) than in the 55.9% fat matrix (7.22±0.31 min). The D-value of *Salmonella* in the 87% fat formulation (11.08±0.36 min) was significantly higher (P<0.05) than in other formulations.

Significance: Since significantly higher D-values for *Salmonella* in low a_w foods may result at elevated temperature, food composition and temperature dependence of a_w should be considered when validating thermal inactivation processes.

P2-161 Development of a Real-time Food Pathogen Detection Platform Using Immunoassay Nanoparticle Technology

MIHO MATAKATSU[1], Kiyoshi Yamaki[2], Hideyuki Tanaka[2], Kazuo Haga[2], Michael Weber[1], John Coomes[1] and John Bodner[1]

[1]Toho Technology Inc., Chicago, IL, [2]Toho Technology, Inazawa, Aichi, Japan

Introduction: The rapid and accurate detection of pathogens present in food or environmental samples provides food producers critical information to implement corrective actions while minimizing production down time. Real-time pathogen measurement during enrichment culture is one approach to improving the time to actionable results particularly for low level foodborne pathogen samples.

Purpose: The purpose of this study is to demonstrate the performance of a direct pathogen measurement system using a nanotechnology-based immunoassay coupled with Surface enhanced Raman scattering (SERS) detection methodology. The system has been designed not only to reduce the time of detection as wells as to minimize the risk of pathogen exposure to the user running the system and the surrounding environment.

Methods: The surface of 60nm colloidal gold nanoparticleis coated with a Raman activere reporter molecule (SERS-Tag). The SERS-Tag is then conjugated to pathogen specific antibodies while magnetic microparticles are coupled with complementary antibodies to the same pathogen target. The target pathogen is immunologically bound by both the SERS-Tag nanoparticle and the magnetic particle. Aggregates form a large magnetic pellet that can be pulled to the side of a culture vessel where the pellet is interrogated with a laser at defined wavelength.

Results: Pathogen specific SERS nanoparticles and pathogen specific magnetic particles were directly added to the selective media. These conditions did not interfere with the growth kinetics of the target pathogen. Direct measurement of the resulting Raman spectral signal corresponded to the amount of pathogen present in the sample. The limit of detection was 1 CFU per food sample. Analytical sensitivity was typically 10^5CFU/mL.

Significance: The results obtained in this study support that SERS nanotechnology-based immunoassay coupled with real-time measurement during enrichment is a powerful method for rapid detection of foodborne pathogen.

P2-162 Production and Characterization of Monoclonal Antibodies Highly Specific to Peanut Protein

JEONG SOOK KIM[1] and Won Bo Shim[2]

[1]Gyeongsang National University, Jinju, Korea, Rebublic Of, [2]Gyeongsang National University, Jinju, South Korea

❖ ***Developing Scientist Competitor***

Introduction: *Food allergy presents a problem for many parts of society, including sensitive subjects, schools, health authorities, and the food industry. The peanut is one of the major food allergens, and a rapid detection method for peanut adulteration is necessary to protect consumers who suffer from peanut allergy.*

Purpose: The objectives of this study are to confirm a thermal-stable soluble protein (TSSP) in peanut, and to develop and characterize monoclonal antibodies (MAbs) specific to peanut using TSSP as an immunogen.

Methods: TSSP in peanut was extracted by non-heating and heating treatment and confirmed by SDS-PAGE. TSSP was used as an immunogen, and the mice showing high titer were used for cell fusion and cloning. The characterization of MAbs produced from hybridoma cells obtained were performed by indirect ELISA and Western blot.

Results: SDS-PAGE protein pattern with raw peanut extracted by non-heating treatment showed major protein bands at 100 kDa, 65 kDa and 10 to 40 kDa, whereas the roasted, boiled peanuts and peanut butter extracts obtained by heating treatments showed major bands at 65 kDa and 10 to 40 kDa. Six mice were immunized with roasted or boiled peanut extracts obtained by heating treatment, and four MAbs (RP 5F9-23, RP 6G4-30, RP 4C12-10 and BP 3A1-12) were developed. The RP 5F9-23, RP 6G4-30 and BP 3A1-12 MAbs were confirmed to be a specific MAb to peanut without cross-reaction to other nuts and food allergen, whereas the RP 4C12-10 MAb was reacted soybean in the indirect ELISA and Western blot analyses. The ELISA based on the MAbs assay can sensitively detect 0.005 % peanut protein.

Significance: These results support that the MAbs developed can be sufficiently used as a bio-receptor in the development of immunoassay for the simple and rapid detection of peanut in food products.

P2-163 Detection of Pork Fat in Heat-processed Beef Meat Products by ELISA Using Monoclonal Antibody Specific to Pork Fat Protein

JEONG SOOK KIM[1] and Won Bo Shim[2]

[1]Gyeongsang National University, Jinju, Korea, Rebublic Of, [2]Gyeongsang National University, Jinju, South Korea

❖ ***Developing Scientist Competitor***

Introduction: Meat and food products containing pork meat and fat may cause serious matter for a Muslim, Jewish and for vegetarians. Therefore, detection and identification of pork adulterated in meat products and food is concerned as an urgent need.

Purpose: The objective of this study was to develop and optimize an indirect ELISA using anti-pork fat monoclonal antibody (MAb) for the rapid detection of pork fat and its application to the detection of pork fat in heat-processed beef meat products such as autoclaved, steamed, roasted and fried meat.

Methods: To improve the sensitivity of the indirect ELISA, the optimal sample pre-cooking time, sample coating time and temperature, primary and secondary antibody dilution time were tested. Various buffer systems including sample dilution buffer and blocking buffer were optimized. Heat processed beef meat samples artificially adulterated with known amounts of pork fat (0, 1, 5, 10, 15, 30, and 100%, w/w) were prepared by autoclaving, steaming, roasting and frying and analyzed by the indirect ELISA.

Results: A standard curve was obtained based on the optimized conditions and the developed indirect ELISA method can sensitively detect 1 % pork fat in heat-processed (autoclaving, steaming, roasting and frying) beef meat. No cross-reaction to other meats such as beef, chicken, goat, duck, horse, and sheep was not obtained in the direct ELISA system.

Significance: In order to detect and identify pork adulteration in meat products and food is of concern to vegetarians and some religions such as Islam and Judaism. These results indicated that the indirect ELISA can be a useful tool for the rapid screening and quantification of pork fat adulteration in meat products.

P2-164 ELISA Screening Assays for Florfenicol and Fluoroquinolones

RONALD SARVER, Brent Steiner, Meaghan Sherry, Douglas MacRae, Danielle Delamarter and John Heller
Neogen Corporation, Lansing, MI

Introduction: Aquaculture is a major source of seafood for human consumption, although chemicals used to treat and prevent disease can lead to accumulation of chemical residues. Few animal antibiotics are approved for aquaculture, so rapid screening methods are needed to detect drug residues in seafood.

Purpose: Enzyme-linked immunosorbent assays (ELISAs) were developed to screen for florfenicol and fluoroquinolones in animal tissues. Performance of the ELISAs were validated according to 2002/657/EC guidelines and results reported.

Methods: Following 2002/657/EC guidelines for screening assays, validation for florfenicol screening was completed by collecting ELISA data on extracts from 60 different Salmonidae muscle samples spiked with 1,000 ppb florfenicol and 60 negative controls. Use for avian muscle was then validated by collecting ELISAs on extracts from 20 avian muscle samples spiked with 150 ppb florfenicol and 20 negative controls. Similarly, validation of the extraction and ELISA for fluoroquinolones was completed in shrimp and avian muscle.

Results: Mean ELISA results were 1,474 ± 473 ppb for 1,000 ppb florfenicol spiked Salmonidae muscle samples, 11 ± 19 ppb for unspiked, 199 ± 87 ppb for 150 ppb spiked avian muscle and 12 ± 12 ppb for unspiked. Three of 60 spiked Salmonidae and 1 of 20 of the spiked avian muscle samples were false compliant. Mean ELISA results were 0.90 ± 0.22 ppb for 1.0 ppb enrofloxacin spiked shrimp samples, 0.15 ± 0.09 ppb for unspiked, 1.16 ± 0.25 ppb for spiked avian muscle samples, and 0.50 ± 0.13 ppb for unspiked. One of 60 enrofloxacin spiked shrimp samples and 0 of 20 enrofloxacin spiked avian muscle samples were false compliant. All data sets conformed to 2002/657/EC criteria for screening methods.

Significance: To help ensure animal protein products are not contaminated with drug residues, validated ELISA screening methods are now available for detection of florfenicol and fluoroquinolones in animal tissues.

P2-165 ELISA Screening Assays for Tetracycline and Sulfonamides

RONALD SARVER, Brent Steiner, Meaghan Sherry, Douglas MacRae, Danielle Delamarter and John Heller
Neogen Corporation, Lansing, MI

Introduction: Increasing resistance of bacteria to antibiotics is a public health concern and certain antibiotics are not permitted in food animals. There is a need for rapid drug residue screening methods for use in production facilities, processing plants, and testing laboratories.

Purpose: Enzyme-linked immunosorbent assays (ELISAs) were developed to screen for sulfonamides and tetracyclines in animal tissues. Performance was validated according to 2002/657/EC guidelines and results were reported.

Methods: Following 2002/657/EC guidelines for screening assays, validation for sulfonamide screening was completed by collecting ELISA data on extracts from 60 different bovine kidney samples spiked with 100 ppb sulfadimethoxine and 60 negative controls. Use for avian muscle was then validated by collecting ELISA results for extracts from 20 different avian muscle samples spiked with 100 ppb sulfadimethoxine and 20 negative controls. Similarly, validation of the extraction and ELISA for tetracyclines was completed in porcine and avian muscle tissue.

Results: Mean ELISA results were 71.3 ± 37.5 ppb for 100 ppb sulfadimethoxine spiked kidney samples, 2.2 ± 2.6 ppb for unspiked, 81.5 ± 25.6 ppb for spiked avian muscle samples and 1.0 ± 1.0 ppb for unspiked avian samples. Two of the 60 sulfa spiked kidney samples and none of the spiked avian muscle samples were false compliant. Mean ELISA results were 9.9 ± 2.3 ppb for 10.0 ppb tetracycline spiked porcine muscle samples, 0.2 ± 0.9 ppb for unspiked, 6.7 ± 2.0 ppb for spiked avian muscle samples and 0.01 ± 0.04 ppb for unspiked samples. Three of the 60 tetracycline spiked porcine muscle samples and none of the tetracycline spiked avian muscle samples were false compliant. All data sets conformed to 2002/657/EC criteria for screening methods.

Significance: To help ensure animal protein products are not contaminated with drug residues, validated ELISA screening methods are now available for detection of sulfonamide and tetracycline in animal tissues.

P2-166 Comparison of Manual Assurance GDS and Assurance GDS Pickpen PIPETMAX Procedures for Preparation of Challenging Food and Environmental Samples

Philip Feldsine, Khanh Soliven, Khyati Shah, Joseph Berry and **TIM KELLY**
BioControl Systems, Bellevue, WA

Introduction: Preparation of samples for analysis by Assurance GDS methods involves sample enrichment, reagent dispensing, immunomagnetic separation using the proprietary PickPen device, and sample transfer steps. The Assurance GDS PickPen PIPETMAX has been customized to perform an automated alternative to prepare samples for Assurance GDS analysis. This was introduced in 2016. This year the focus is on especially difficult matrices.

Purpose: Consistent with last year, to compare the equivalence of sample preparation for Assurance GDS analysis performed manually using the PickPen device and in an automated manner utilizing the Assurance GDS PickPen PIPETMAX (PPMX) instrument equipped with a PickPen head in a variety of new and challenging foods by multiple Assurance GDS methods.

Methods: A total of 323 samples of various matrices were analyzed by multiple Assurance GDS methods. Inoculated samples were enriched according to kit directions for use. Paired samples were taken. One set was prepared manually while the other set was processed by the PPMX. Both sets of prepared samples were analyzed on the BioControl Rotor-Gene Q instrument. Positives were confirmed and matched for the two sample comparative preparation methods and Ct (amplification cycle threshold) values compared for all positive samples.

Results: A total of 375 samples were tested. 274 samples were confirmed positive with both the manually processed and those prepared using the PPMX automated system. 101 samples were negative by both methods. There were no discrepant results. The mean Ct values across all the positive curves were within 0.3 of each other. Four different Assurance GDS assays were evaluated. In total, five different foods and three surfaces were tested. They are known to be challenging due to factors such as heavy particulates, inhibitory materials, high microbial load.

Significance: This validation study demonstrates the equivalence of the Assurance GDS sample preparation method comparing manual processing with the new automated system, the Assurance GDS PickPen PIPETMAX for a growing number of difficult food matrices and surfaces.

P2-167 Genetic Characterization Based on Four Housekeeping Genes of Sixteen Human-Pathogenic *Bacillus* Species Isolated from Foods, Cosmetics, and Environmental Surveillance Samples

IRSHAD SULAIMAN[1], Ying-Hsin Hsieh[1], Emily Jacobs[1], Steven Simpson[1] and Khalil Kerdahi[2]
[1]*U.S. Food and Drug Administration, Atlanta, GA,* [2]*U.S. Food and Drug Administration, Aylanta, GA*

Introduction: The genus *Bacillus* is a group of Gram positive, rod shaped, human pathogenic bacteria known to cause foodborne disease worldwide. This surveillance study was carried out to determine the presence of *Bacillus* spp. in foods, drugs, and cosmetics and, thereby, ensure that these commodities are safe and can be consumed by American people.

Purpose: The major objective of this study was rapid detection and differentiation of *Bacillus* spp. of public health importance by DNA sequencing of four housekeeping genes.

Methods: A total of 50 isolates belonging to 16 *Bacillus* spp. (*Bacillus amyloliquefacians, Bacillus cereus, Bacillus circulans, Bacillus coagulans, Bacillus firmus*, *Bacillus laterosporus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus mycoides, Bacillus pumilus, Bacillus smithii, Bacillus sphaericus, Bacillus subtilis, Bacillus thuringiensis,* and *Bacillus vallismortis*) were recovered from food, cosmetic, and environmental samples by enrichment followed by streaking onto selective media. Subsequently, sequence analysis was performed for all recovered isolates at the rRNA (ribosomal RNA), *gyr*B (DNA gyrase β subunit), *rpo*B (RNA polymerase β subunit) and *tuf* (TU elongation factor) loci using ABI 3500XL Genetic Analyzer.

Results: Species specific genetic polymorphism was observed for *Bacillus* spp. at the four housekeeping genes characterized. Nevertheless, the *tuf*, *gyr*B, and *rpo*B genes provided more resolution as compared to the 16S rRNA sequences. Thus, these three genes may be considered an efficient alternative target for identification and taxonomic analysis of *Bacillus* spp.

Significance: The results suggested that genetic discrimination for *Bacillus* spp. can be achieved by sequence characterization of the regions of rRNA, *gyr*B, *rpo*B, and *tuf* genes.

P2-168 Real-time Monitoring of TVC Using Non-invasive Bioluminescence Growth Media

Ryan Marder, Brandon Katz, Paul Meighan and **DELIA CALDERON**
Hygiena, Camarillo, CA

Introduction: This study introduces a new technology from Hygiena, MicroSnap Surface Express, for the real time monitoring for non-specific viable bacteria from surfaces. The collected samples begin to produce light as they metabolise a new substrate, indicating by the emission of light per bacteria a new method to both enumerate and detect at low CFUs. CFU burden collected proved that the method can be used as a disaster test to indicate very rapidly if a heavy bioburden of viable bacteria was swabbed.

Purpose: To demonstrate performance of MicroSnap Surface Express Total in the field as an easier and simpler method for detecting viable bacteria from environmental samples in a single, self-contained device.

Methods: 47 sites within a food plant were sampled. then split into 2 portions: one portion was incubated with the real time viability growth media and the second portion inoculated onto agars to count TVC. MicroSnap Surface Express devices were incubated at 30C and measured for bioluminescence each hour for 12 hours. At 24 hours the TVC plates were counted and compared to each hour in the bioluminescent growth cycle.

Results: The results show that increasing bacteria from the surfaces produced detection in shorter incubation times. The detection was inversely proportional to the CFU, with shorter incubation periods of 1 to 5 hours detection in the first bin (>5000 CFU), as levels of bacteria swabbed decreased the time to result increased The following are mean time (n=5 to 10) for the following bins 1001 to 5000 CFU time to result <6 hours, 101 to 1000 time to result <7hours, 11 to 1000 <8 hours and <10 in 9 hours.

Significance: The new method equips food processors with an ultra-rapid tool for identifying viable bacteria as part of a sanitation monitoring program.

P2-169 Optimizing Methods for Recovering Heat-injured *Enterococcus faecium* and Indigenous Enterococci in Turkey Litter Compost

HONGYE WANG, Zhao Chen and Xiuping Jiang
Clemson University, Clemson, SC

❖ ***Developing Scientist Competitor***

Introduction: In order to validate the physical heat treatment applied to eliminate *Salmonella* spp. in poultry litter as biological soil amendments, there is a need to use a surrogate strain for the pathogen. *Enterococcus faecium* NRRL B-2354 has been used as a *Salmonella* surrogate in thermal process validation studies. Since bacterial cells may be injured during heat treatment, an effective recovery step should be incorporated to allow the resuscitation of heat-injured cells for an accurate enumeration.

Purpose: The objective of this study was to optimize the recovery method for isolating heat-injured indigenous enterococci and desiccation-adapted *Salmonella* surrogate *E. faecium* from turkey litter compost.

Methods: The turkey litter compost samples with and without inoculation of *Salmonella* surrogate *E. faecium* NRRL B-2354 (desiccation-adapted) in the Tyvek pouches were exposed to 75°C for up to 1 h. Recovery media for heat-injured indigenous enterococci were bile esculin agar (BEA) or enterococcosel agar (EA); modified two-step overlay (OV) method (OV/BEA and OV/EA); and modified thin agar layer (TAL) method (TAL/BEA and TAL/EA). To recover heat-injured *E. faecium*, BEA and EA, in the above recovery media, were supplemented with rifampin (R; 100 µg/ml).

Results: The initial populations of *E. faecium* and indigenous enterococci in turkey litter compost were 8.21 and 5.45 log CFU/g, respectively. Among those six recovery methods, OV/EA-R plates yielded the highest populations (P< 0.05) of *E. faecium* (5.09±0.33 log CFU/g). For heat-treated turkey litter compost without inoculation, background microorganisms growing on OV/EA, OV/BEA, TAL/EA, and TAL/BEA caused interference with the enumeration of indigenous enterococci. Therefore, BEA and EA were selected to recover the heat-injured indigenous enterococci.

Significance: The selected recovery methods for heat-injured *E. faecium* and indigenous enterococci can be used in the thermal process validation studies on poultry litter.

Poster

P2-170 Evaluation of a Novel Method for Detection of *Enterobacteriaceae* in Dairy Infant Formula Using Real-time PCR

LAURENT JAIN[1], André Quintanar[2], Jean-Philippe Tourniaire[1], Sophie Pierre[1] and Jean-François Mouscadet[1]
[1]*Bio-Rad, Marnes-la-Coquette, France,* [2]*Bio-Rad, Hercules, CA*

Introduction: *Enterobacteriaceae* (EB) are found in many food matrices; among them dairy infant formula and related environmental sources. Their absence is required since they can cause serious illness in babies. While isolation by culture can be a labor intensive process, a real-time PCR method could allow rapid qualitative detection, especially in the presence of competing free DNA and flora.

Purpose: This study evaluated inclusivity/exclusivity, LDi, and performance of a novel qPCR-based method for detecting this large family of bacteria in different infant formula and associated ingredients.

Methods: The test includes five steps: enrichment, Free DNA Removal Solution (FDRS), DNA extraction, real-time PCR, and data interpretation. For inclusivity/exclusivity testing, 282 certified strains at ~10^5 CFU/ml or DNA were tested (including 248 belonging to the EB family). The LDi was determined by using a 10-fold serial dilution of a *Salmonella* spp. strain from equivalent 7.4 x 10^6 to 0.74 CFU/ml. To evaluate the method sensitivity, 17 different matrices (12 milk powders, 3 with probiotics, and 5 ingredients) were inoculated at 5 CFU/ml (or not). All samples were culture confirmed following the ISO 21528-1:2004 method. Cq values for both EB and internal control targets were analyzed.

Results: The assay demonstrated 100% inclusivity for EB and 100% exclusivity for non-EB with the collection of strains evaluated. LDi results were confirmed to 68 CFU/ml. For effectiveness testing, concordance to culture confirmation was 100% for all PCR assays, even on probiotic formula enriched with vancomycin.

Significance: This study indicates that the iQ-Check® *Enterobacteriaceae* in combination with the FDRS is a rapid and sensitive method for detecting EB in dairy infant formula and ingredients prone to the presence of dead cells. Test kit results demonstrated no significant difference when compared with the reference culture method.

P2-171 Inhibition of Bacterial and Plant AB Toxins by Polyphenolic Compounds

BEATRIZ QUIÑONES
U.S. Department of Agriculture-ARS-WRRC-PSM Unit, Albany, CA

Introduction: The AB-type protein toxins elicit a cytotoxic effect by inhibiting protein synthesis. AB-type toxins include Shiga toxins from *Escherichia coli* and ricin from the plant *Ricinus communis*. Several methods have been developed to detect the toxin-induced inhibition of protein synthesis but are limited by complex and laborious processing steps.

Purpose: Given the limitations of current methods, the present study develop a simple, rapid and quantitative cell-based assay to detect the inhibition of protein synthesis by AB-type toxins.

Methods: A Vero cell line, Vero-d2EGFP, was constructed to constitutively express a destabilized variant of the enhanced green fluorescent protein (EGFP). This destabilized EGFP variant was employed as a sensitive marker for measuring the inhibition of protein synthesis. To screen for natural compounds that can inactivate AB toxins, grape extracts were subjected to separation and purification to different degrees of polymerization by using high-performance liquid chromatography.

Results: Incubation with ricin reduced the Vero-d2EGFP fluorescence in a dose-dependent manner. The loss of EGFP fluorescence was much more dramatic than the loss of cell viability: a half-maximal effective ricin concentration (ED50) of 0.03 ng/mL was recorded by the Vero-d2EGFP assay, whereas cell viability assay reported an ED50 of 0.7 ng/mL. The Vero-d2EGFP assay was then employed to screen for toxin inhibitors. A significant loss of fluorescence was recorded for Vero-d2EGFP cells challenged with Shiga toxins in the presence of fractions 1-6, composed of mostly polyphenolic monomers and dimers. However, intoxicated cells co-incubated with fraction 7, composed of mostly polyphenolic tetramers, retained a stronger fluorescent signal, representing a statistically significant difference from the intoxicated control Vero-d2EGFP cells (P = 0.0217, Student's t test).

Significance: These results highlight that the Vero-d2EGFP assay is highly sensitive and enables the rapid detection and inactivation of AB5 toxin activity with minimal sample handling for data acquisition.

P2-172 Assessing Biological, Chemical, and Radionuclide Detection Methods

PENNY NORQUIST[1] and John Larkin[2]
[1]*FPDI, Saint Paul, MN,* [2]*University of Minnesota and Food Protection and Defense Institute, St. Paul, MN*

Introduction: Numerous methods to detect intentional adulteration of biological, chemical, or radionuclide agents in food have been developed. Determining which method is appropriate for a specific application depends on knowing the capabilities/limitations of the method compared to a set of standardized acceptable levels of usage and performance.

Purpose: Establish a detection method assessment model for intentional adulteration agents for use in method suitability selection and determination of needed method improvements.

Methods: Experts from industry, government and academia through both large workshops and small subgroups, identified the important attributes for assessing the suitability of a method, along with specifications for levels of Outstanding, Excellent, Satisfactory, and Limited for each attribute for both biological and chemical methods. The radionuclide group developed a subset of these attributes more applicable for radionuclides.

Results: The experts specified 23 attributes. As an example, the performance specification for Sensitivity [POD 9c)] is: Limited > ± 10%, Satisfactory ± 1-10%, Excellent < ±1% and Outstanding <±0.1%. The levels are mostly contiguous, while Outstanding often sets a level of potential future attainability. A database of over 560 citations on research and commercial detection methods was developed. A user can conduct searches by agent and type of method. The selected attributes of the resulting search can then be displayed to visually see differences in the methods level performance. An draft assessment tool was presented at the BioWorld Defense Summit in May 2016. This talk will discuss additional research completed on radionuclides and application of the assessment tool to determine research gaps and compare methods for all agent types.

Significance: A user can use the assessment criteria to set criteria for a method supplier. A method supplier can determine where improvements are needed for detection of a specific agent. The process used for intentional adulteration methods can be transformed into an assessment method for food safety applications.

P2-173 Evaluation of Potential Temperature Abuse on Different Meat Packaging Systems during Outdoor Cooking Events

MARGARET JACKS[1], Luxin Wang[1] and Thomas Taylor[2]
[1]Auburn University, Auburn, AL, [2]Varcode, Wellesley, MA

Introduction: When meat products are stored improperly at temperatures between 4°C and 60°C, bacteria counts can double every twenty minutes. Unfortunately, not all consumers are aware of the danger zone or do not avoid it. One reason is the lack of easy-to-use measurement tools to help them monitor temperature abuses.

Purpose: The purpose of this study was to monitor potential temperature abuses during outdoor cooking events with the use of two temperature monitoring tools.

Methods: Overwrapped or vacuum packaged meats were purchased from a local market and used in this study. Freshcode™ strips were attached to the front and the back of each package. Three storage conditions were used: meats placed in an ice chest with ice, a closed ice chest without ice under the sun, and on a counter top at room temperature. The strips were read every hour for eight hours and the data was compared to corresponding traditional thermometer readings. Total aerobic plate counts and coliform counts were determined at the beginning and the end of each trial. Both the overwrapped and vacuum packaged meats underwent the same conditions and data collection procedures.

Results: The monitoring efficiency of the Freshcode™ strips varied among the different packages. There was no significant difference between the readings generated by the strips and the traditional thermometer for overwrapped packaging systems ($P > 0.05$). The strips applied to the vacuum packaged meats registered the temperature change an hour slower than the overwrapped packages ($P \geq 0.05$). The total aerobic plate and coliform counts increased by approximately one Log CFU/g of meat under each storage condition.

Significance: Easy-to-use temperature monitoring methods are needed by consumers to better evaluate potential temperature abuses during outdoor cooking events. Commonly used ice chests do not completely prevent temperature abuse. Different packaging systems may need different monitoring methods.

P2-174 Performance of a Rehydratable Film Medium for the Quantitative Enumeration of Lactic Acid Bacteria

MARA CELT, Adam Stanenas, Robert Jechorek, John David and Cari Lingle
3M Food Safety, St. Paul, MN

Introduction: Lactic acid bacteria (LAB) are an essential component for fermented foods, and these organisms have been known to benefit the human digestive systems as a probiotic. However, they can be key spoilage organisms in foods causing off flavors, discoloration, and bloating. There are numerous traditional agar methods for this large group of microorganisms including ISO 15214 and several methods in the Compendium of **Methods** for the Microbiological Examination of Foods. These agar reference methods are laborious, and may require anaerobic chambers for the most efficient growth environment. To increase efficiency of LAB testing by eliminating agar preparation, a new rehydratable film method has been developed with a self-contained anaerobic environment. The new rehydratable film method can also distinguish between homofermentative and heterofermentative LAB.

Purpose: This study compared two agar reference methods to a new rehydratable media method for the detection and enumeration of LAB.

Methods: Test portions from 17 matrices were prepared by adding 11 g of each matrix to 99 mL of diluent. Samples were homogenized before serial dilutions were performed. One mL of diluted sample was plated, in duplicate, onto Petri dishes and the new rehydratable film method, 3M™ Petrifilm™ Lactic Acid Bacteria Count Plates. Tempered MRS agar was added to samples in Petri dishes and allowed to solidify at room temperature. If specified in the reference method, agar plates were placed in anaerobic jars with gas packs. Agar plates were incubated at the appropriate temperature, as outlined in the reference method. The rehydratable media method was incubated at 28°C and 37°C.

Results: The rehydratable media method was not statistically different (Mean Log Difference ≤ 0.5 logs) from the two reference methods tested.

Significance: The new rehydratable film method is an acceptable alternative to the most commonly used LAB agar methods.

P2-175 Extended Spectra Database for Quality Indicators and Other Spoilers Identification By MALDI-TOF: A Never-Ending Story…

Marian Awad, **DANIÈLE SOHIER**, Simone Becker and Markus Kostrzewa
Bruker Daltonics, Bremen, Germany

Introduction: Indicator organisms are employed to reflect the microbiological quality of foods and beverages. They are most often used as a measure of the hygienic or sanitary conditions, but they are as well used to describe an organism whose presence or level indicates the potential for future spoilage or as a "surrogate" organism; for example, those organisms that model the behavior of pathogens under certain conditions. In addition, some "indicators" are not specific organisms but assays for groups of organisms, such as *Enterobacteriaceae* (EB).

Purpose: Opening lines to develop the database is to focus on groups of micro-organisms, one after another. This helps in creating a road map, starting with the most commonly encountered then also moving to spoiler species linked to more specific industrial processes or formulations.

Methods: In that step-by-step database implementation program, isolates from dairy, meat and malt fermentation processes and strains collections were characterized using both 16S rDNA sequencing and Maldi-Tof Mass Spectrometry (MS). A minimum of 20 MS Profiles (MSPs) were obtained for each isolates to set up a Reference MSPs. Ideally, whenever possible, three different isolates or more are analyzed per species.

Results: The global database contains currently 104 species of lactic acid bacteria, species of 155 *Enterobacteriaceae*, 396 species of the main spore-forming bacteria spoilers including *Alicyclobacilliaceae*, *Bacilliaceae*, *Paenibacilliaceae* and *Clostridia,* and as well 193 yeasts. Blind isolates were analyzed and were accurately identified and distinguished from over 7311 other microorganisms present in the overall database.

Significance: The integration of Maldi-Tof MS combined to a fit-for-purpose database into existing food and beverage production processes helps to improve quality assurance practices, providing accurate identification and short-time to result. This high-throughput routine platform is as well a promising technology to identify quickly the origins of product spoilage, or to develop a strategy for quality indicators profile's and other spoiler's surveillance.

P2-176 Isolation and Characterization of Wide Host Range-specific Bacteriophage for the Development of a Magnetoelastic Biosensor Method

DO HYEON PARK

Kyungpook National University, Daegu, South Korea

Introduction: Magnetoelastic biosensors have been considered one of the most promising and rapid detection methods. Bacteriophages are required as a bio-recognition element for the development of the magnetoelastic biosensor method.

Purpose: The purpose of this study was to isolate, purify, and characterize a wide host range-specific (WHR) bacteriophage as a new bio-recognition element.

Methods: WHR bacteriophages that were isolated from a poultry processing plant using dot and single plaque assays were purified through PEG precipitation, CsCl ultra-centrifugation, and dialysis. The selectivity of the WHR bacteriophages was investigated against 44 foodborne pathogens using dot assays. Morphological characteristics of the WHR bacteriophages were confirmed using a TEM. The stability of the WHR bacteriophages was investigated by incubating for 1 h under varying pH (3, 4, 5, 7, 9, 11, and 12) and temperature (4, 10, 22, 37, 50, and 60°C) conditions. Gel-electrophoresis was performed, after the extraction of the DNA from WHR bacteriophages, to determine the size of its DNA.

Results: The WHR bacteriophages were isolated from water used in poultry processing plants, and its final concentration was determined to be 5.81×10^{11} PFU/mL. Clear zones were found against six foodborne pathogens including two strains of *Escherichia coli* O157:H7, *Bacillus cereus, Aeromonas hydrophila*, *Pseudomonas aeruginosa,* and *Salmonella* Heidelberg. TEM images of the WHR bacteriophages confirmed that it had a head (131 ± 2.70 nm) and a tail (169 ± 8.51 nm). The optimum pH and temperature of the WHR bacteriophages were found to be in the ranges of 4.0-11.0 and 4-37°C, respectively. Gel-electrophoresis indicated that the WHR bacteriophage consisted of dsDNA with a genome size of 23-27 kb.

Significance: The WHR bacteriophages were successfully isolated and purified, and this study showed that the WHR bacteriophages had sufficient possibility to be used as a bio-recognition element for the development of the biosensor method.

P2-177 A Single Laboratory Validation for the Microbial Identification of *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* Utilizing MALDI-TOF Technology

MICHAEL BROWN[1], Kristopher Stanya[1], Nichelle Kunecke[1], Lauren May[1], Ken Yoshitomi[2] and Lisa Newberry[1]

[1]U.S. Food and Drug Administration, Bothell, WA, [2]U.S. Food and Drug Administration, Rockville, MD

Introduction: Recent advances in mass spectrometry have resulted in a new generation of stand-alone systems for bacterial identification using MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization Time-of-flight Mass Spectrometry) technology.

Purpose: This study evaluated the performance of the VITEK® MS microbial identification system with regard to rapid identification of food associated bacterial isolates. These instruments have the capability to significantly reduce identification timeframes for unknown bacterial isolates from hours to minutes when compared to the current, validated identification systems (VITEK® 2, API®).

Methods: A randomized, blinded bacterial panel consisting of 232 traceable, previously characterized environmental and reference isolates consisting of *Salmonella* spp. (*n*=102), *Escherichia coli* (*n*=50), *Listeria monocytogenes* (*n*=50), and various exclusionary organisms (*n*=30) were analyzed in parallel using both the VITEK® MS and the VITEK®2 systems.

Results: The results of this study indicate that the VITEK® MS produced equivalent identification of the three major bacterial species, 96%, 98% and 99% agreement for *L. monocytogenes*, *E. coli* and *Salmonella* spp., respectively, when compared to the VITEK® 2 reference method. Additionally, the VITEK®MS system did not identify any of the exclusionary organism panel as one of the three target pathogens or as any other species.

Significance: Single laboratory validation results support further evaluation in a multi-laboratory study, with the goal of routine implementation of the VITEK® MS for rapid pathogen identification from food samples.

P2-178 Evaluation of Enumeration and MPN Prediction Methods for *Staphylococcus aureus*

JENNIFER HAIT and Sandra Tallent

U.S. Food and Drug Administration, College Park, MD

Introduction: Staphylococcal food poisoning is one of the most prevalent causes of foodborne intoxication worldwide. As such, the use of enumeration procedures for *Staphylococcus aureus* continues to be essential in order to monitor the safety and quality of food products. The most probable number (MPN) method is utilized for routine surveillance of products in which small numbers of *S. aureus* are expected or in foods predicted to contain a large population of competing species.

Purpose: The research objective was to evaluate culture methods for the detection and isolation of *S. aureus,* including testing different pre-enrichment broths, plated media, and the automated TEMPO instrument for the enumeration of *S. aureus* from assorted foods.

Methods: Seven different food commodities, including two naturally contaminated foods, were evaluated at levels ranging from 10-10^5CFU/g of inoculated target and non-target organisms. In total, 80 food samples were analyzed. Duplicate test portions of 25g each were homogenized with 225mL of Butterfield's phosphate buffer (1/10). Five tube MPN predictions comparing two pre-enrichment broths, TSB containing 10% NaCl and 1% sodium pyruvate (PTSBS), and Giolitti and Cantoni (GC) were evaluated alongside the TEMPO STA and the direct plate count method.

Results: Food analysis and the data demonstrated that the PTSBS pre-enrichment media for MPN prediction of *S. aureus* was more selective and less cumbersome to use and provided more accurate result interpretation than the GC pre-enrichment broth, with 80% proportion of overall agreement. Results for the TEMPO STA proved comparable to the reference method for *S. aureus*, with 90% proportion of overall agreement.

Significance: The study indicated the PTSBS performed better, overall, when evaluating accuracy and ease of use once compared to the GC broth for MPN prediction of *S. aureus*. Furthermore, the TEMPO STA yielded results in 24 hours that were comparable to the current four day enumeration method.

P2-179 Suitability of ATP Bioluminescence Compared to pH Measurement or Microbial Growth on Agar to Evaluate Commercial Sterility in UHT Milk

Cristian Morano[1], Marcela Smichth[1], Luciana Maiorano[2], **MICHELE FONTANOT**[3] and Gabriela Stancanelli[2]

[1]Sucesores de Alfredo Williner S.A., Rafaela - Santa Fe, Argentina, [2]3M Argentina, Buenos Aires, Argentina, [3]3M Peru SA, Lima, Peru

Introduction: Rapid technologies, such as microbial-ATP detection have focused on detecting microbial contamination in shorter times than traditional methods, allowing a faster time to release of Ultra-High Temperature (UHT) processed products. The use of ATP bioluminescence is a rapid alternative method. Argentina regulations require pH measurement of UHT milk after seven days of incubation at 35°C for product release. Some bacteria may not reduce pH after seven days of incubation and may hamper the microbial quality of this type of product.

Purpose: This study evaluated the reliability of using an ATP bioluminescence method and pH measurement to screen for commercial sterility.

Methods: ATP bioluminescence, pH and agar methods were used to evaluate 120 samples. Three different products (*n*=40/product) were tested; whole, low fat and chocolate milk. A set of 60 samples was incubated for 48h at 35°C and an additional 60 samples at 72h at 35°C for enrichment. Forty-five samples were spiked with ≤10 CFU *Pseudomonas aeruginosa* and 45 with ≤10 *Bacillus cereus.* Positive controls were spiked with 100 CFU and 30 samples were not spiked.

Results: Samples spiked with Pseudomonas did not present a pH decline, however it was detected by growth on agar and ATP bioluminescence. Samples spiked with Bacillus showed a limited pH decline after 48h and was, also, detected by growth on agar and ATP bioluminescence. Microbial contamination was not determined in uninoculated samples evaluated with any of the three methods.

Significance: Bioluminescence technology provides a rapid alternative for evaluating commercial sterility in UHT milk and has offers UHT manufacturers a reliable alternative for product release with equivalent results to determination of microbial growth on agar and with better reliability than pH measurement.

P2-180 Development of Loop-mediated Isothermal Amplification (LAMP) for Detection of BW10KD Allergen in Buckwheat

DONG JOO SEO[1], Hyunkyung Park[2], Suntak Jeong[2], Hanseam Shin[3] and Changsun Choi[4]

[1]Chung-Ang University, Food & Nutrition, Anseong, Kyounggi, South Korea, [2]Chung-Ang University, Anseong, South Korea, [3]Chung-Ang University, Ansung, South Korea, [4]Chung-Ang University, Ansung, Kyounggi, South Korea

Introduction: Buckwheat has been commonly consumed in Asian countries, including Korea and Japan, because it has been recognized as functional food to prevent hypertension and cardiovascular disease. However, BW10KD, a 10 kDa buckwheat protein, is known as one of the major allergenic proteins in buckwheat. The contamination of buckwheat by the allergen has potential to induce serious allergenic reaction in sensitized people. A polymerase chain reaction (PCR) assay to detect the BW10KD gene, which encodes the allergenic proteins of buckwheat was recently developed.

Purpose: Since the ELISA and PCR assays for BW10KD were not sensitive enough to detect buckwheat contamination in food, this study aimed to develop a rapid and reliable loop-mediated isothermal amplification (LAMP) technique for its detection.

Methods: This study developed a BW10KD-specific LAMP detection assay, with high specificity and sensitivity, for use in foods. DNA was extracted from buckwheat, beans, wheat, and rice purchased from wholesale market. The sensitivity and specificity of LAMP and PCR for BW10KD were compared in buckwheat, bean, wheat, and rice DNA.

Results: The sensitivity of LAMP was 10-times higher than PCR. The detection limit of LAMP for BW10KD was as low as 100 pg of buckwheat BW10KD. LAMP developed in this study could specifically detect BW10KD without cross-activity with other food allergen.

Significance: This study concluded that LAMP is a rapid and reliable technique to detect the contamination of buckwheat allergen in foods.

P2-181 Strategy for Quantification of *Staphyloccous aureus* Enterotoxins from Foodborne Intoxication Cases by Mass Spectrometry

MIRJANA ANDJELKOVIC[1], Sarah Denaeyer[1], Nadine Botteldoorn[1] and Andreja Rajkovic[2]

[1]Scientific Institute of Public Health, Brussels, Belgium, [2]Ghent University, Ghent, Belgium

Introduction: Food poisoning caused by ingestion of *Staphylococcus aureus* enterotoxins (SET) is one of the most common foodborne-diseases and most of the SETs may induce symptoms by doses as low as 20-100ng per kg body weight. Since SET are thermostable, pH resistant and protease resistant proteins (20-30kD) they can withstand pasteurisation, food processing techniques and the acidic stomach environment.

Purpose: In the frame of microbial food safety risk assessment establishing the toxic dose easily measurable by using mass spectrometry prior to consumption is a big concern. This study provides specific and accurate analytical tools for detection and quantification of these toxins.

Methods: Whereas for A, B, C (C1, C2, C3), D and E serotype commercial kits are available other serotypes may only be indirectly concluded after PCR measurements. For the purpose of the study six *S.aureus* H serotype producers from foodborne intoxications were identified and selected. With the extraction method recommended by the European Reference Laboratory for Coagulase positive Staphylococci, toxins were extracted from the *S.aureus* bacteria cultured on different growth media double Brain-Heart Infusion followed by the trypsin digestion of the extracted toxins (proteins) allowing the detection of its proteotypic peptides.

Results: Trypsin digests of enterotoxin (6 *S.aureus* isolates x 4 cell density x 3 growth phases x 3 replicates, including positive and negative control) were screened by liquid chromatography coupled to tandem mass spectrometry for the presence of the toxin using selected proteotypic heavy-labelled peptides as internal standards. The toxins were quantified in the extracts coming from the *S.aureus* isolates at the level of 4ng/g

Significance: LC-MS/MS approaches can be used for the detection and quantification of other SETs involved in foodborne outbreaks or clinical cases (eg asthma related extracts) and in risk assessment to determining the dose of SETs associated with toxicity.

P2-182 Validation Report for the Method Comparison Evaluation of the bioMérieux TEMPO Aerobic Count (AC) to the Health Canada MFHPB-18 Determination of the Aerobic Colony Counts in Foods Reference Method for the Enumeration of Viable Mesophilic Aerobic Bacteria in a Variety of Foods

PATRICK BIRD, James Agin, Benjamin Bastin, Joe Benzinger, Erin Crowley and David Goins

Q Laboratories, Inc., Cincinnati, OH

Introduction: The enumeration of the total aerobic mesophilic flora is used to determine the sanitary quality of finished products and can indicate its state of freshness or decomposition. The TEMPO® AC (Aerobic Count) is an automated test for use with TEMPO for the enumeration of total aerobic mesophilic flora within 22-28 hours in a wide variety of food products.

Purpose: The purpose of this study was to conduct a method comparison evaluation of the bioMérieux TEMPO AC to the Health Canada MFHPB-18 reference method. The results of this study were collected according to Annex 3.1 of the Health Canada Compendium and submitted to the Health Canada Microbiological Methods Committee (MMC) in order to obtain the "all foods" claim for mesophilic aerobic bacteria for subsequent publication in the HC Compendium of Analytical Methods .Methods: The study consisted of analyzing five food categories, with one food type from each category being evaluated in a paired study design. Each food type was evaluated for naturally occurring mesophilic bacteria . The target range of contamination was achieved by diluting one lot of the matrix with high naturally occurring mesophilic bacterial counts with one lot of the matrix that produced lower counts in order to produce the target range of contamination. The validation study included evaluating 30 paired twenty-five (25) gram replicate samples in duplicate at six different levels of contamination.

Results: For each matrix evaluated, no statistically significant differences were determined between the alternative and reference methods.

Significance: Based on the results of the statistical analysis, the data generated in the evaluation support the claim that the TEMPO AC method is a comparable method to the MFHPB-18 and meets the requirements for the "all foods" claim for mesophilic aerobic bacteria. This method has been approved for publication in the HC Compendium of Analytical Methods.

P2-183 Investigation of Resonant Mass Measurement for Physiological Analysis of Microorganisms

BYRON BREHM-STECHER and Hyun Jung Kim
Iowa State University, Ames, IA

Introduction: Information on microbial physiology has traditionally been obtained using imaging methods such as light, fluorescence and electron microscopy. Other methods, such as flow cytometry, can provide data on dynamic physiological changes occurring in microbial populations with single-cell resolution. However, physiological properties such as mass are typically measured using bulk-phase techniques, which yield values that are averaged across entire populations. Filling this gap, the LifeScale-R instrument (Affinity Biosensors, LLC) enables flow-through mass measurement of individual microbes using resonant cantilever technology.

Purpose: We sought to explore use of resonant mass measurement for physiological characterization of stress-filamented *Salmonella* spp. and for early detection of germ tube formation in *Candida albicans*.

Methods: Five *Salmonella* serovars and *E. coli* (non-*Salmonella* control) were filamented under conditions of salt- or cold-stress and reversion of filaments to smaller, individual cells was monitored over time after removal of these stressors. Mean mass and mass distribution of *Salmonella* samples were monitored and compared with forward- and side-scatter data collected via flow cytometry. For analysis of *Candida albicans*, yeast cells were inoculated into rich media containing serum and were measured over time to determine if mass-based measurements could enable early detection of germ tube emergence. Non-C. *albicans* germ tube-negative control strains were also examined.

Results: Mean mass and mass distribution measurements made using the LifeScale-R clearly highlighted the post-stress physiological differentiation of *Salmonella* filaments into smaller, individual cells. LifeScale-R data correlated well with parallel light scatter data collected using flow cytometry. For *C. albicans*, incubation in germ tube-inducing media resulted in increasing cell mass as a function of time, with visible elongation of cells, however, these cells proved too large for successful analysis in the LifeScale-R as it is currently configured.

Significance: This study demonstrates the utility of resonant mass measurement for single-cell analysis of dynamic changes in microbial physiology.

P2-184 Evaluation of the Performance of an Alternative Rapid Molecular Detection Assay Based on Loop-mediated Isothermal Amplification (LAMP), Compared to a Reference Official Mexican Method (NOM 210), in Artificially Contaminated Alkaline-treated Corn Meal (Nixtamal)

ILSE GARCÍA and Ismael Espinosa
3M, Ciudad de Mexico, Mexico

Introduction: *Listeria monocytogenes*, is a widely recognized pathogen associated with foods, leading to illness and death of vulnerable populations including pregnant women, immunocompromised adults and children under 5 year of age. *L. monocytogenes* surveillance and its monitoring in raw ingredients and environment is pivotal to prevent contamination and its detection is often a requirement for product release.

Purpose: To evaluate the performance of a LAMP based rapid method for the detection of *Listeria monocytogenes* in alkaline treated corn meal compared with Official Mexican Method (NOM210) that is based on ISO11290.

Methods: Five different lots of nixtamal meal were used. For each lot 25 g portions were inoculated with 1 to 5 CFU/portion of *L. monocytogenes (ATCC 19115)* and analyzed with the LAMP-based method (*n*=30 samples/lot with additional *n*=10 samples/lot of uninoculated samples) or with the NOM210 method (*n*=2 samples/lot with additional *n*=2 samples/lot of uninoculated samples). All samples were enriched with 225mL of Demi Fraser broth for 24h at 35°C. Samples analyzed with the NOM210 method were further confirmed through biochemical tests. Analysis of results was done using an unpaired design.

Results: Inoculated samples from all lots resulted in positive detection of *L. monocytogenes* by both LAMP-based and NOM210 methods. For all analyzed samples (N=220), no positive or negative deviations were determined and the LAMP-based method was found to be equivalent to the NOM210.

Significance: The alternative LAMP-based molecular detection assay can offer a specific and rapid approach for the detection of *L. monocytogenes* that can be implemented as part of the microbiological quality test for release test of nixtamal meal.

P2-185 Rapid Detection of *Cronobacter* spp. in Powdered Infant Formula Related Products, Raw Materials, and Environmental Samples Utilizing Loop Mediated Isothermal Amplification (LAMP) and Bioluminescence Detection Technologies

Christina Barnes, Neil Percy, **CYNTHIA ZOOK**, Lisa Monteroso and Gabriela Lopez Velasco
3M Food Safety, St. Paul, MN

Introduction: For newborns and infants, *Cronobacter* spp. are opportunistic pathogens. Outbreaks associated to *Cronobacter* spp. have been linked to the consumption of powdered infant formula (PIF). Manufacturing environment, raw material, and final product for these pathogens are under strict surveillance by PIF manufacturers and health organizations to prevent illness.

Purpose: This study compared the performance of a LAMP-bioluminescence based method and the ISO 22964 (2006) standard for the detection of *Cronobacter* spp. from a variety of PIF manufacturing related products and environmental samples.

Methods: Inclusivity (106 *Cronobacter* spp. strains) and exclusivity (102 non-*Cronobacter* spp. strains) were assessed following AOAC guidelines. Method comparison was performed with 10-375 g portions of PIF (*n*=162), environmental samples (*n*=190), cereals (*n*=84), and raw materials (*n*=138) including mineral-vitamins, fatty acids, oils, flours, and probiotics. Samples were spiked with ≤ 5 CFU/portion of lyophilized *Cronobacter* spp. cultures. Portions were enriched with buffered peptone water ISO at 37±1°C for 18-24h. Detection was done by the LAMP-Bioluminescence assay. Enrichments were further confirmed following the ISO 22964 standard. The results from 10 g portions (*n*=319) were analyzed according to ISO 16140-2 and portions >10 g (*n*=255) were analyzed by Chi-square. Additionally, the probability of detection in 300 g portions and limit of detection were evaluated.

Results: Inclusivity and exclusivity were determined to be 100% for the LAMP-bioluminescence assay. Performance of the assay showed that there was no significant difference compared to the ISO-22964 standard. The LOD of the assay was 10^3CFU/mL in the enrichment and the lowest concentration detected was 1-2 CFU/300g portions.

Significance: The alternative LAMP-bioluminescence method offers a specific and rapid approach for the detection of *Cronobacter* spp., offering PIF manufacturers and commercial laboratories a next day result to evaluate the microbiological quality of these products.

Poster

P2-186 Evaluation of the New USDA Neutralizing Buffered Peptone Water Formulation for Poultry Verification Samples

PATRICIA RULE[1], Stan Bailey[2], Deborah Briese[2], Vikrant Dutta[1], John Mills[2], Ron Johnson[1] and Jeff Papi[1]
[1]bioMérieux, Inc., Hazelwood, MO, [2]bioMerieux, Inc., Hazelwood, MO

Introduction: USDA FSIS Notice 41-16 details the use of a new formulation of Buffered Peptone Water (BPW) that now includes neutralizing agents that should minimize the impact of antimicrobial interventions on target organism recovery for poultry verification testing.

Purpose: This study was a two-part investigation. The first phase of the study examined the efficacy of using the automated Masterclave® to prepare the neutralizing Buffered Peptone Water (nBPW) for routine laboratory use. Phase two was a comparison study between the USDA reference method for poultry rinses and the GENE-UP® *Salmonella* assay using the nBPW from phase one.

Methods: The nBPW was made per USDA media preparation and autoclave parameter instructions substituting the Masterclave for the generic autoclaving step. A spiking study was then performed comparing the SLM method to the USDA/FSIS MLG 4.09 for *Salmonella* isolation using the nBPW previously prepared. Thirty samples per method were analyzed for this comparison study. This included five uninoculated, five high (2 -5 CFU/test portion) and twenty low (0.2-2 CFU/test portion) samples. All analytical outcomes were biochemically confirmed regardless of the PCR screening result.

Results: All five uninoculated samples were confirmed negative for both methods and all five high samples confirmed positive for both methods. For the low level inoculation samples, the PCR method had six confirmed positive, whilethe reference method yielded seven positives. The dPOD and the corresponding 95% confidence interval is -0.05 (-0.32;0.23). This indicates no statistically significant difference between the two methods evaluated during this study and the efficacy of the Masterclave.

Significance: These data demonstrate that the Masterclave is a suitable method for the preparation of the new USDA nBPW formulation. These data also confirm that there are no performance issues when using this nBPW medium when testing with the GENE-UP PCR system.

P2-187 Comparison of Swabbing, Rinsing, and Grinding as Sampling Methods for the Recovery of Indicator Microorganisms on Beef Trimmings

MANSOUR ALNAJRANI, Andrea English, Keelyn Hanlon, Alejandro Echeverry and Mindy Brashears
Texas Tech University, Lubbock, TX

Introduction: Many sampling methods have been developed to assess the microbial quality of beef products. However, a little is known about the impact of different sampling methods on final counts for beef trimmings.

Purpose: To examine the effectiveness of several sampling methods for the recovery of microbial indicators in beef trim.

Methods: Fifteen samples of beef trimmings were collected from an establishment using the N60 technique under federal inspection. Each sample was subjected to multiple sequential sampling methods including swabbing, rinsing, and grinding of the same trim sample divided among the sampling methods. A 100 cm2 of beef trimming was swabbed three sequential times with sterile sponges pre-hydrated buffered peptone water (BPW). 25g of beef trimmings were rinsed three times sequentially in BPW. Finally, 25g of trimmings was ground and the ground meat was rinsed three times in BPW. Appropriate serial dilutions were plated onto aerobic plate count and *E. coli* petrifilms and incubated at 36°C±1 for 24 and 48 hours.

Results: Generic *E. coli* counts were below the detection limit (<10CFU/ml) for all of the sampling methods and samples. The aerobic bacterial counts obtained from the first enumeration of swabbing, rinsing and grinding were 1.9 logCFU/100 cm2, 2.9logCFU/g and 3.0logCFU/g, respectively. The total aerobic bacteria recovery using swabbing, rinsing, and grinding were 2.3logCFU/100cm2, 3.0logCFU/g, and 3.1logCFU/g, respectively. Swabbing was significantly lower than rinsing and grinding (P<0.05) for both the first or total bacterial recovery. However, the total coliform recovery using rinsing was not significantly different (P<0.05) from either swabbing or grinding, but swabbing recovered the least. Simple linear regression showed a moderate correlation of 0.74 and 0.64r2 for swabbing vs rinsing and rinsing vs grinding methods, respectively.

Significance: The efficacy of the sampling method used is critical in evaluating the microbiological quality of beef trimmings to improve public health.

P2-188 Quantitative Fluorometric Detection of *Escherichia coli* in Ground Beef Using Genetically Engineered Bacteriophages

ANQI CHEN
Cornell University, Ithaca, NY

✦ ***Undergraduate Student Award Competitor***

Introduction: Conventional methods carried out for *Escherichia coli* detection, one of the major pathogens, require long time for bacterial growth followed by a series of time-consuming and complicated procedures. Fortunately, determination of β-galactosidase (β-gal), an intercellular enzyme released by *E. coli*, is an ideal alternative for *E. coli* detection. The release of β-gal can be achieved by introducing bacteriophage (phage) to infect and lyse the *E. coli* bacterial cells.

Purpose: This study investigated detection of *E. coli* in drinking water using genetically modified T7 bacteriophages with 4-Methylumbelliferyl-β-D-galactopyranoside (MUG) as substrate. The product from MUG cleaved by β-gal is fluorescent, therefore detectable by fluorescence spectroscopy.

Methods: Four factors were considered to optimize *E. coli* detection: MUG concentration, growth media (PBS buffer vs. LB broth), control vs. engineered phage, and temperature (25°C vs. 37°C). Under optimized conditions, T7 genetically engineered phage and MUG were added to *E. coli* cultures with the following concentrations (10^5, 10^4,10^3,10^2, and 10^1 ,CFU/mL) plus the negative control group. The fluorescence intensity was measured every hour through 8 hours for the determination of the *E. coli* detection limit.

Results: The optimized condition included 2μM MUG, PBS buffer, engineered phage, and 37°C. It was found that the concentration of *E. coli* cells is critical to the fluorescence response: higher *E. coli* concentration required less detection time. *Escherichia coli* cells at the concentration of 10^5, 10^4,10^3,10^2, 10^1 CFU/mL were detectable after total detection times of 5,6,7, and 8 hours, respectively. The fluorescence intensity data was represented as the average of three independent experiments.

Significance: This is a rapid and facile approach for examining the presence of *E. coli* in drinking water when compared to conventional methods. It can also be applied to the food industry for microbiological testing to ensure food safety.

Poster

P2-189 Thermo Scientific™ SureTect™ *Escherichia coli* O157:H7 Assay: NF Validation Using the 7500 Fast PCR Instrument

Emma Scopes[1], Ana-Maria Leonte[1] and **AMANDA MANOLIS**[2]
[1]Thermo Fisher Scientific, Basingstoke, United Kingdom, [2]Thermo Fisher Scientific, Austin, TX

Introduction: The Thermo Scientific™ SureTect™ *Escherichia coli* O157:H7 PCR Assay is a real-time PCR assay for the detection of *E. coli* O157:H7 from raw beef, which has previously gained NF Validation by AFNOR Certification using the Thermo Scientific™ SureTect™ PikoReal™ PCR instrument and Thermo Scientific SureTect™ Software version 1.2.

Purpose: The purpose of this study was to conduct an NF Validation by AFNOR Certification extension study to validate use of the SureTect *Escherichia coli* O157:H7 PCR Assay on the Applied Biosystems™ 7500 Fast PCR Instrument with Applied Biosystems™ RapidFinder™ Express version 2.0 Software (the alternative method) with seasoned and unseasoned raw beef meats.

Methods: A method comparison study and relative limit of detection (RLOD) study was conducted using the SureTect™ standard protocol as the alternative method. Twenty five g samples were enriched in 225 ml of pre-warmed (41.5±1°C) BPW and incubated at 41.5±1°C. Direct lysis was conducted following 8 and 24 hours enrichment. Following direct lysis, PCR was run and results were automatically interpreted by the software. The reference method was conducted according to ISO 16654:2001.

Results: Sixty raw beef samples were tested using the alternative and ISO 16654:2001 reference methods. For the RLOD study, an isolate of *E. coli* O157:H7 was spiked into a raw beef trim matrix and tested according to the alternative method protocol and the ISO 16654:2001 reference method. The alternative method demonstrated equivalent performance for the raw beef meat samples analysed to the ISO 16654:2001 reference method. The alternative method showed the same RLOD (0.4-0.7 CFU/25 g) to the ISO 16654:2001 reference method (0.4-0.7 CFU/25 g) at both 8 and 24 hours incubation.

Significance: The alternative method proved to be a suitable substitute to the ISO 16654:2001 reference method for *E. coli* O157:H7 detection in seasoned and unseasoned raw beef meats.

P2-190 Thermo Scientific™ SureTect™ *Listeria monocytogenes* Assay: NF Validation Using the 7500 Fast PCR Instrument

Emma Scopes[1], Ana-Maria Leonte[1] and **AMANDA MANOLIS**[2]
[1]Thermo Fisher Scientific, Basingstoke, United Kingdom, [2]Thermo Fisher Scientific, Austin, TX

Introduction: The Thermo Scientific™ SureTect™ *Listeria monocytogenes* PCR Assay is a real-time PCR assay intended for the detection of *L. monocytogenes* from food products and environmental samples, which has previously gained NF Validation by AFNOR Certification using the Thermo Scientific™ SureTect™ PikoReal™ PCR instrument and Thermo Scientific™ SureTect™ Software version 1.2.

Purpose: The purpose of this study was to conduct an NF Validation by AFNOR Certification extension study to validate use of the SureTect™ *Listeria monocytogenes* PCR Assay on the Applied Biosystems™ 7500 Fast PCR Instrument with Applied Biosystems™ RapidFinder™ Express version 2.0 Software (the alternative method) using meat products, milk and dairy products, seafood and fishery products, vegetables, plus environmental samples.

Methods: A method comparison study and relative limit of detection (RLOD) study was conducted. For the alternative method, all samples underwent an enrichment step followed by direct lysis. Following direct lysis, PCR was run and results were automatically interpreted by the software. The reference method was conducted according to EN ISO 11290-1/A1:2004.

Results: A total of 393 food and environmental samples (78 meat products, 78 milk and dairy products, 65 seafood and fishery products, 75 vegetables, plus 97 environmental samples) were tested using the alternative and EN ISO 11290-1/A1:2004 reference methods. For the RLOD study, five different *L. monocytogenes* isolates were spiked into representative matrices and tested as per the alternative method protocol and the EN ISO 11290-1/A1: 2004 reference method. The alternative method demonstrated equivalent performance for all human food and environment samples analyzed as per the EN ISO 11290-1/A1:2004 reference method. The alternative method showed an RLOD (0.2-1.0 CFU/25 g) similar to that of the EN ISO 11290-1/A1:2004 reference method (0.3-0.9 CFU/25 g).

Significance: The alternative method proved to be a suitable substitute to the EN ISO 11290-1/A1:2004 reference method for *L. monocytogenes* detection.

P2-191 Thermo Scientific™ SureTect™ *Salmonella* Species Assay: NF Validation Using the 7500 Fast PCR Instrument

Emma Scopes[1], Ana-Maria Leonte[1] and **AMANDA MANOLIS**[2]
[1]Thermo Fisher Scientific, Basingstoke, United Kingdom, [2]Thermo Fisher Scientific, Austin, TX

Introduction: The Thermo Scientific™ SureTect™ *Salmonella* species PCR Assay is a real-time PCR assay intended for the detection of *Salmonella* species from food and environmental samples, which has previously gained NF Validation by AFNOR Certification using the Thermo Scientific™ SureTect™ PikoReal™ PCR instrument and Thermo Scientific SureTect™ Software version 1.2.

Purpose: The purpose of this study was to conduct an NF Validation by AFNOR Certification extension study to validate use of the SureTect *Salmonella* species PCR Assay on the Applied Biosystems™ 7500 Fast PCR Instrument with Applied Biosystems™ RapidFinder™ Express version 2.0 Software (the alternative method) with meat products, milk and dairy products, powdered infant formula, raw beef meats, seafoods and vegetables, plus environmental samples.

Methods: A method comparison study and relative limit of detection (RLOD) study was conducted. For the alternative method, all samples underwent an enrichment step followed by direct lysis. Following direct lysis, PCR was run and results were automatically interpreted by the software. The reference method was conducted according to ISO 6579:2002.

Results: Three hundred ninety-one food and environmental samples (72 meat products, 62 milk and dairy products, 64 powdered infant formula, 61 raw beef meats, 63 seafood and vegetables, plus 69 environmental samples) were tested using the alternative and ISO 6579:2002 reference methods. For the RLOD study, five *Salmonella* spp. isolates were spiked into representative matrices and tested as per the alternative method protocol and ISO 6579:2002 reference method. The alternative method demonstrated equivalent performance for the food and environment samples analysed to the ISO 6579:2002 reference method. The alternative method showed a similar RLOD (0.4-1.5 CFU/25 g) to the ISO 6579:2002 reference method (0.4-1.3 CFU/25 g).

Significance: The alternative method proved to be a suitable substitute to the ISO 6579:2002 reference method for detection of salmonellae.

P2-192 Detection of *E. coli* O157:H7 and *Salmonella* in a Cannabis Simulant Using a Liquid Crystal-based Immunoassay

CURTIS STUMPF[1], Brian Bullard[1], Stephanie Kuzenko[1], Emily Rusnak[1] and Gary Niehaus[2]
[1]*Crystal Diagnostics Ltd., Rootstown, OH,* [2]*Northeast Ohio Medical University, Rootstown, OH*

Introduction: Patients using medicinal cannabis often have compromised immune systems. Thus, *E. coli* O157:H7 and *Salmonella,* which can contaminate cannabis and cannabis-based products, pose a significant health risk to these patients. To mitigate this risk, the current project developed a liquid crystal-based screening test for detection of *E. coli* O157:H7 *and Salmonella* in raw cannabis. The rapid screening (<16 hours to results) of a simulant of raw cannabis was developed using a single-stage enrichment.

Purpose: A liquid crystal-based assay was developed, tested, and compared against culture-based methods for detection of *E. coli* O157:H7 and *Salmonella* in a cannabis simulant.

Methods: A simulant of cannabis, hops (*Humulus lupulus*), was inoculated with low levels (<10 cells) of *E. coli* O157:H7 and *Salmonella* and permitted to stabilized overnight. The samples were then enriched in mTSB+n and mTSB, respectively, for 15-24 hours. Detection was conducted in a liquid crystal-based detection platform and compared against cultures of the processed antibody-coated microspheres on Chromagar O157 (*E. coli* O157:H7) and XLT4 and Hektoen enteric agar (*Salmonella*).

Results: For *E. coli* O157:H7 testing, the liquid crystal-based assay correctly detected 96% (*n*= 23) of the culture positive samples and 93% (*n*= 15) of the non-inoculated or fractionally negative samples. For *Salmonella*, the assay correctly detected 92% (*n*= 25) of the culture positive samples and 96% (*n*= 25) of the non-inoculated and fractionally negative samples. Detection between the liquid crystal-based platform and the culture-based analysis showed similar results at low levels of inoculation.

Significance: This liquid crystal-based immunoassay provides a simple one-step enrichment and screening method for detection of *E. coli* O157:H7 and *Salmonella* in cannabis.

P2-193 Withdrawn

P2-194 Detection and Survival of Viable But Non-culturable *Escherichia coli* O157 in Soil

CALLUM HIGHMORE and Charles William Keevil
University of Southampton, Southampton, United Kingdom

Introduction: Contaminated fresh produce is responsible for more disease cases than any other food commodity, yet current detection methods for foodborne pathogens overlook cells in the viable but non-culturable (VBNC) state. It has been shown that the VBNC state can be induced in the foodborne pathogen *Escherichia coli* O157, while on the lettuce phylloplane. Additionally, the expression of the pathogen's Shiga-like toxin genes has been observed in VBNC *E. coli* O157, suggesting that it can remain pathogenic despite being non-culturable.

Purpose: This study aimed to develop a direct method of detection of foodborne pathogens in soil including VBNC cells, overcoming the shortcomings associated with molecular methods when analyzing complex environmental matrices.

Methods: Bacterial adherents were released from soil particles into suspension using a Pulsifier and concentrated by two rounds of vacuum filtration, reducing 25 g of soil to a 1 ml suspension. *Escherichia coli* O157 was quantified in eleven soil samples by qPCR, targeting the *tir* (translocated intimin receptor) gene. Cells were elongated via incubation with pipemidic acid and visualized through PNA-FISH under epifluorescence microscopy.

Results: The assay was capable of detecting *E. coli* O157 at 10 CFU per gram of peat-based compost. The *tir* gene was detected in seven of eleven soil samples tested, despite disparate compositions and geographical origins. Peat-based compost contained the highest concentration, at 200 gene copies per gram. The presence of VBNC *E. coli* O157 was confirmed in the compost using PNA-FISH.

Significance: The assay described here provided a rapid and quantitative pre-screen of foodborne pathogens in soil, with applications in determining the extent of their presence throughout the environment. VBNC populations of *E. coli* O157 have been observed within compost, indicating its presence in agricultural soils across the UK.

P2-195 Single Marker Detection and Virulence Gene Profiling of STEC in Produce and Associated Farmscape Samples

JANNETH PINZON[1], Kamieko Kayoshi[1], Jeremy Roland[1], Adrian Sbodio[1], Bettina Groschel[2], William Chaney[2], Erin Dreyling[3], Michele Jay-Russell[4] and Trevor Suslow[1]
[1]*University of California-Davis, Davis, CA,* [2]*Roka Bioscience, San Diego, CA,* [3]*Roka Bioscience, Warren, NJ,* [4]*University of California-Davis, Western Center for Food Safety, Davis, CA*

Introduction: Available commercial systems designed for rapid presumptive screening for shiga-toxin producing *E. coli* target the *stx* and *eae* genes. Strains of clinical concern contain alternative mechanisms for virulence missed by reliance on the cardinal *eae* marker. Environmental STEC isolates containing less than the prerequisite complement of virulence traits are routinely detected on produce and the associated farmscape environment.

Purpose: To further evaluate the potential utility of single marker technology for the detection of non-O157 STEC in the produce production environment and to investigate the confirmatory presence of virulence genes in purified isolates.

Methods: 177 samples (basil, bok choy, leeks, kale, chard, collard, parsley, soil, sediments, and irrigation water) were collected in four seasonal samplings. Bacteria were captured by filtration using Modified Moore Swabs and enriched in Tryptic Soy Broth-novobiocin, and incubated for 24 h at 42°C. Enrichments were screened with the Atlas® STEC EG2 Combo Detection Assay and cultural isolation conducted. A multiplex MXPCR for *E. coli 16s rDNA*, *stx1*, *stx2,* and *eae* gene targets was used for initial characterization. Positive strains were examined by PCR for seven virulence-associated genes. Known clinical non-O157 were reference standards.

Results: 52 (29.4%) samples were positive for STEC. Ten samples (5.6%) were positive for *E. coli* O157:H7. Basil showed a higher positive outcome (10.2%). Of 373 presumptive *E. coli* colonies purified, only 7 isolates (1.9%) were *stx*/*eae* positive, 34 (9.1%) and 44 (11.8%) were individually positive for *stx* and *eae*, respectively. Seventeen different marker profiles were obtained. Most of the STEC were isolated from positive enrichments, however isolates carrying *stx* and other virulence genes (e*hxA, adfO)*were recovered from negative samples.

Significance: This study contributes to a larger longer-term effort to clarify the risk associated with the diverse STEC group and its application to routine compliance and lot acceptance testing for fresh produce.

Poster

P2-196 Simultaneous Enrichment of *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* from Environmental Swabs and Detection by Multiplex-qPCR

ASHLEY QUEEN[1], Kirsten Hirneisen[1], Venugopal Sathyamoorthy[2], Atin Datta[2] and Donna Williams-Hill[1]
[1]U.S. Food and Drug Administration, Irvine, CA, [2]U.S. Food and Drug Administration - CFSAN, Laurel, MD

Introduction: *Escherichia coli O157:H7 (EHEC), Salmonella spp.(S), and Listeria monocytogenes (Lm)* are the most significant pathogens with respect to FDA regulated food products. Standard methods for environmental swab sample analysis target only one pathogen in a sample by preparation of multiple pre-enrichment media according to the Bacteriological Analytic Manual; this is time consuming, and cost/labor intensive. Current research has identified candidate universal enrichment broths and methods for detection via real-time multiplex qPCR (mqPCR); therefore a rapid and accurate method to simultaneously enrich and detect multiple targets would provide a critical reporting tool.

Purpose: This study is a collaborative effort to identify a universal enrichment broth for the simultaneous enrichment of *EHEC*, *S*, and *Lm* from environmental swab samples.

Methods: A plastic tub, was marked with large (4x4 inch) and small (1x1 inch) grids for swabbing per AOAC. Duplicate grids were spiked with 100ul of high (HS) or low (LS) BHI broth culture concentrations of *EHEC* ($1.82x10^{8}$ CFU/ml; $1.82x10^{5}$ CFU/ml), *S* ($1.62x10^{8}$ CFU/ml; $1.62x10^{5}$ CFU/ml), and *Lm* ($2.66x10^{8}$ CFU/ml; $2.66x10^{5}$ CFU/ml). Large and small surface area swabbing was performed using Dey-Engley broth moistened sponge-tipped swabs and cotton-tipped swabs respectively; followed by two-hour room temperature incubation, twenty-four hour enrichment in LEB broth at 35ºC, DNA extraction, and mqPCR.

Results: mqPCR analysis detected multiple microorganisms in the HS grids in both the large and small surface areas. *Lm* and *EHEC* were detected with Ct values of 25.74±1.09 and 31.79±1.58, respectively, in LEB broth enrichment from the HS large grid sample (n=2). There was a significant difference for *Lm* detection in HS of large vs. small surface areas (P<0.02), despite equal amounts of spiked organisms.

Significance: A universal enrichment broth to simultaneously enrich multiple bacterial pathogens would enhance assessment of processing facility sanitation while using fewer resources and reducing reporting time by 24-48 hours.

A Comparative Evaluation of the GENE-Up Assay for the Detection of *Escherichia coli* O157:H7

VIKRANT DUTTA[1], Mick Bosilevac[2], Hari Dwivedi[1], John Mills[3], Patrice Chablain[4] and Stan Bailey[3]
[1]bioMérieux, Inc., Hazelwood, MO, [2]U.S. Department of Agriculture–ARS, Clay Center, NE, [3]bioMerieux, Inc., Hazelwood, MO, [4]bioMérieux, Marcy, France

Introduction: Shiga-toxigenic *Escherichia coli* O157:H7 (STEC O157) remains one of the major food-borne public health threats since it was first identified as a pathogen in 1982. In the US, per year, the CDC estimates ca. 95,000 STEC O157 infections, among which ca. 3700 are lab confirmed cases. STEC O157 contaminated beef and beef products are now considered adulterated by the FSIS. The current PCR systems remain inherently burdened with cumbersome sample preparation, especially for complex matrices such as beef and beef products, along with complex instrumentation and interpretation tools. The GENE-UP™ *E. coli* O157:H7 (ECO) is a Fluorescence Resonance Energy Transfer (FRET) based real-time PCR that utilizes probe-melt peak analysis to detect STEC O157 while simultaneously differentiating between *E.coli* O157:H7 and O157:non H7.

Purpose: To perform a comparative evaluation of ECO methods against the USDA/FSIS MLG 5.09 and 5A.04.

Methods: In total, 451 mTSB enriched beef and beef products samples were processed through MLG and ECO. All positive results were confirmed using culture-based isolation of STEC O157. Any discrepant results were retested for confirmation.

Results: Comparative sensitivity, specificity, false positive rate, and false negative rate against culture between MLG and ECO were 91 vs 92, 91 vs 99%, 9 vs 0.76%, and 8.5 vs 8.3%, respectively. Positive predictive value, negative predictive value and the overall accuracy were found to be 54 vs 95, 99 vs 99%, and 91 vs 99%, for MLG and ECO, respectively.

Significance: These data demonstrate that the ECO assay provides a highly sensitive detection of *Escherichia coli* O157:H7 that is comparable to USDA/FSIS MLG. The convenient sample preparation along with a user friendly instrumentation and software interface, makes the GENE-UP™ *E. coli* O157:H7 a viable alternative for *Escherichia coli* O157:H7 detection.

P2-198 Development of Sample Preparation Methods to Improve Multiplex PCR Performance for Detection of *Escherichia coli* on Leafy Vegetables

YUKYUNG CHOI[1], Jiyoung Lee[1], Heeyoung Lee[1], Sanghyun Han[2] and Yohan Yoon[1]
[1]Sookmyung Women's University, Seoul, South Korea, [2]Microbial Safety Team, Agro-Food Safety & Crop Protection Department, National Institution of Agricultural Science, Rural Development Administration, Wanju, South Korea

❖ ***Developing Scientist Competitor***

Introduction: Pathogenic *Escherichia coli* is one of the major cause for foodborne outbreaks in Korea, and the pathogen has been detected from leafy vegetables. To detect *E. coli*in leafy vegetables, a multiplex PCR method as rapid detection method is used, but its detection limit is too high.

Purpose: The purpose of this study was to find sample preparation methods to improve multiplex PCR performance to detect *E. coli*in leafy vegetables.

Methods: A five strain-mixture of *E. coli* was inoculated in leafy vegetables (cabbage, lettuce and sprouts) at 1, 2 and 3 log CFU/g. The inoculated samples were placed in sample bags containing buffered peptone water (BPW), followed by hand shaking for 30 times. The homogenates were then subjected to control, centrifugation (1912×g at 4ºC for 15 min), filtration (0.45 μm), and enrichment with *Escherichia coli* broth at 44.2ºC. Pellets from centrifugation and filters from filtration were resuspended in 5 ml BPW. One-milliliter aliquots from the resuspensions and enriched broth were used to extract DNA, and then multiplex PCR method was performed with the DNA.

Results: For lettuce, the filtration and enrichment method (3 h - 24 h) were positive at all inoculation levels but all negative for control and centrifugation method. For cabbage, filtration and enrichment method were positive for all inoculation levels, but centrifugation method was positive only at 3 log CFU/g, and control was negative for all inoculation levels. In sprouts, filtration and enrichment methods were positive at all inoculation level, and centrifugation method was positive at 2 and 3 log CFU/g, but control was positive only at 3 log CFU/g.

Significance: The results indicate that filtration and enrichment methods should be used as a sample preparation method to improve multiplex PCR performance for *E. coli* on leafy vegetables.

Poster

P2-199 High-resolution Melt Curve PCR Assay for Detection of *E. coli* O157:H7 in Beef

YUEJIAO LIU[1], Azlin Mustapha[1] and Prashant Singh[2]
[1]*University of Missouri, Columbia, MO,* [2]*University of Georgia, Griffin, GA*

Introduction: According to the CDC, among the disease-causing Shiga toxin producing *Escherichia coli* (STEC), *E. coli* O157:H7 is estimated to cause one-third of the total STEC illnesses and the most cases of hemolytic uremic syndrome (HUS) in the U.S. The *uid*A gene which is present in the majority of *E. coli* strains, codes for the synthesis of the GUD enzyme. In *E. coli* O157:H7, the *uid*A gene has a single point mutation at the +93 position that leads to an alteration in the amino acid sequence encoding the GUD enzyme.

Purpose: The aim of this study is to distinguish *E. coli*O157:H7 from non-O157:H7 STEC serotypes using a high resolution melt curve (HRM) PCR assay.

Methods: Based on the *uid*A mutation in *E. coli* O157:H7, a reliable PCR assay targeting the *uid*A gene was developed to differentiate *E. coli* O157:H7 from other STECs and the closely related *Shigella* sp. The assay was validated using a set of 120 bacterial strains and spiked ground beef and beef trim.

Results: Isolates of *E. coli* O157:H7 formed distinctive melt peaks that were easily distinguishable from those of non-O157 isolates in the PCR plot. Therefore, this assay was verified to be able to clearly discriminate *E. coli* O157 strains from other *E. coli* and *Shigella*. With a 6-h enrichment, 10 CFU *E. coli*O157:H7 were detectable in 325 g spiked beef samples.

Significance: Accurate detection of *E. coli* O157:H7 is critical in correctly determining the food source of this dangerous pathogen for preventing and controlling foodborne outbreaks.

P2-200 Withdrawn

P2-201 Improvement of Modified Buffered Peptone Water with Sodium Pyruvate (mBPWp) Broth by Optimization of Composition Ratio of Supplements for Rapid Detection of *Escherichia coli* O157:H7

HONG-SEOK KIM[1], Jung-Whan Chon[2], Minjung Shin[1], Dong-Hyeon Kim[1], Young-Ji Kim[1], Il-Byeong Kang[1], Dana Jeong[1], Jin-Hyeong Park[1], Ho-Seok Chang[1], Hyun-Woo Lim[1], Kwang-Young Song[1] and Kun-Ho Seo[1]
[1]*Konkuk University, Seoul, South Korea,* [2]*U.S. Food and Drug Administration/NCTR, Jefferson, AR*

❖ ***Developing Scientist Competitor***

Introduction: In order to ensure food safety and minimize the occurrence of foodborne illness, it is critical to promptly detect causative pathogens in foods. However, there is very little published information on media for shortening enrichment time. Nevertheless, the nucleic acid-based detection methods including PCR are rarely influenced by the number of competing flora.

Purpose: The purpose of this study was to develop customized-enrichment media focused on rapid culture for ideal application of post-enrichment detection technologies.

Methods: As a proof-of-concept study, we optimized modified buffered peptone water with sodium pyruvate (mBPWp; standard medium for enrichment of *Escherichia coli* O157:H7) for rapid culture of *E. coli* O157:H7 (mBPWpR) by comparison of growth curves according to the concentration of each supplement and incubation temperatures (42°C vs. 37°C). The performance of mBPWpR was compared to that of mBPWp for detecting healthy and cold-injured *E. coli* O157:H7 on mixed salad samples.

Results: For rapid culture of *E. coli* O157:H7, the composition of mBPWpR was determined as follows: Lactose was excluded and the concentration of peptone was reduced to 2.5 g/L (25%). The mBPWpR was more effective for initial recovery of *E. coli* O157:H7 in mixed salads, as well as for pure cultures. For instance, at the inoculation level of 10^0 CFU/25g, *E. coli* O157:H7 was recovered from ca. 50% of samples. The cell numbers from the mixed salads were 2.33 ± 0.24 log CFU/mL vs. 1.81 ± 0.22 log CFU/mL in mBPWpR and mBPWp, respectively, after incubation for 8h at 42°C.

Significance: The results of this study showed that mBPWpR could be applied to real-time PCR detection, which is rarely inhibited by competing flora. It was, also, easily validated because the supplements for the broth are identical with those for original mBPWp. The data provided proof-of-concept that enrichment medium enhanced initial recovery of target bacterium and is suitable for rapid detection using PCR technique.

P2-202 Comparing Campy-Cefex with *Campylobacter jejuni/Campylobacter coli* Chromogenic Plating Medium for Isolating *C. jejuni* and *C. coli* from Raw Poultry

PAUL T. NGUYEN, Branislav Basaric, Bill Lionberg and Lawrence Restaino
R & F Laboratories, Downers Grove, IL

Introduction: Greater than 99% of foodborne campylobacteriosis are caused by *Campylobacter jejuni* and *Campylobacter coli*. Campy-Cefex agar (CCA), listed in the USDA Microbiology Laboratory Guidebook for isolating *Campylobacter* from poultry, lacks differentiation for *C. jejuni* and *C. coli* and allows a substantial number of background microbes to grow. In 2012, a *C. jejuni/C. coli* chromogenic plating medium (CCPM) was developed that differentiates these two *Campylobacter* species from other closely related bacteria with an improved selectivity.

Purpose: This study compared CCPM with CCA for isolating *C. jejuni* and *C. coli* artificially inoculated into ground raw chicken and turkey.

Methods: *Campylobacter jejuni* (ATCC BAA-1153), *C. coli* (ATCC 43478), and various microbes used as the background flora were diluted, added to stomacher bags containing raw ground poultry, and hand massaged. Isolation and confirmation of *C. jejuni* and *C. coli* followed the qualitative procedures as outlined in the USDA MLG, with the modification of Bolton broth with selective agents added directly to the 140 poultry meat samples at 1/10 dilution.

Results: At low dilution levels (0.70 to 1.50 CFU/g for *C. jejuni* and 0.70 to 0.84 CFU/g for *C. coli*), CCPM isolated these *Campylobacter* species from 97.5% of the inoculated samples; whereas, CCA detected these microbes in 53.8% of the poultry samples. For inoculation levels ranging from 3 to 8 CFU/g, the incidence of isolation of *C. jejuni* and *C. coli* in the poultry samples were 100.0% and 93.3% for CCPM and CCA, respectively. For CCPM, 83.3% of the positive samples, at low detection levels, required one colony to be picked for confirmation; whereas, 58.5% of the positive samples on CCA were first picked isolates.

Significance: CCPM isolated *C. jejuni/C. coli*, inoculated at low detection levels in raw ground poultry, at nearly twice the frequency compared with CCA. The ease of isolation (detection and plating efficiency, reduce technician time and fatigue, and cost savings) was substantially improved with CCPM.

Poster

P2-203 Evaluation of Selective Enrichment Media and Chromogenic Media for *Salmonella* Detection in Raw Shell Egg Contents with a Low Microbial Load

SOOKYOUNG LEE[1], Kwang-Young Song[1], Jung-Whan Chon[2], Dong-Hyeon Kim[1] and Kun-Ho Seo[1]
[1]Konkuk University, Seoul, South Korea, [2]U.S. Food and Drug Administration/NCTR, Jefferson, AR

❖ ***Developing Scientist Competitor***

Introduction: The main challenge in detecting *Salmonella spp.* in shell eggs is the very low contamination rate, which is estimated to be 1 *Salmonella* positive egg for every 20,000 eggs. Thus, a detection method that is capable of detecting and isolating extremely low *Salmonella* cell numbers in a large volume of liquid eggs is needed.

Purpose: The current study was conducted to evaluate the ability to recover *Salmonella* spp. from shell egg contents by culture methods.

Methods: A total of 4,000 eggs were obtained from grading and packing (GP) centers and 200 samples were created by pooling 20 broken eggs. Pooled samples were held at 25°C for 4 days before 25mL aliquots were added to 225 mL of modified trypticase soy broth (mTSB) and incubated at 35°C for 24 h. A loopfulof overnight broth cultures was streaked onto chromogenic Druggan-Forsythe-Iverson (DIF) agar and incubated for 24 h at 37°C. Also, 1mL and 0.1mL of the culture were added to Muller-Kauffmann tetrathionatebroth with novobiocin(MKTTn) and Rappaport-Vassiliadis Soya (RVS) media, which were incubated for 24 h at 37°C and 42°C, respectively. A loopful of the RV and MKTTn enrichment cultures were streaked onto xylose lysine deoxycholate (XLD), bismuth sulfite (BS), and brilliant green (BG) agar plates.

Results: Directly streaking onto DFI agar revealed the presence of *Salmonella spp.* in 14 of the 200 pooled samples (7%), whereas the combination of RV broth culture and streaking on BG, XLD and BS agar detected the pathogen in only nine (4.5%), seven (3.5%) and three (1.5%) of the pooled samples, respectively. When MKTTn broth was used, *Salmonella* spp. was detected in seven (3.5%), two (1%) and zero (0%) of the samples when streaked onto BG, XLD and BS, respectively.

Significance: The results of this study indicated that the DFI direct plating method without enrichment is the most suitable for the investigation of *Salmonella* spp. in raw shell egg contents with a low microbial load.

P2-204 Addition of Rifampicin to Bolton Broth to Inhibit Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* for the Isolation of *Campylobacter* spp.

KIDON SUNG[1], Jung-Whan Chon[1], Young Ji Kim[2], Young-Jo Kim[3], Ji Young Jung[1], Dongryeoul Bae[1], Saeed Khan[1] and Kun-Ho Seo[4]
[1]U.S. Food and Drug Administration/NCTR, Jefferson, AR, [2]Konkuk University, Seoul, Korea, Republic of (South), [3]Korea Food and Drug Administration, Cheongwon, South Korea, [4]Konkuk University, Seoul, South Korea

Introduction: Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* have become an inhibiting factor for the isolation of *Campylobacter* spp. from chicken products. Rifampicin is broadly active against many Gram positive and some Gram negative aerobic bacteria including ESBL-producing *E. coli*. *Campylobacter spp.* are intrinsically resistant to rifampicin.

Purpose: This study was carried out to check if the addition of rifampicin to Bolton broth improves the isolation of *Campylobacter* spp. in pure cultures and chicken samples.

Methods: The effective concentration of rifampicin (20 µg/mL) for Bolton broth was determined by the MIC test of 40 ESBL-producing *E. coli* collected from different meats against three Bolton broth supplements and rifampicin. In pure culture, five *Campylobacter* strains (each with 10^2cells) were incubated microaerobically in Bolton broth with or without rifampicin at 42°C for 48 h and the number of enriched cells were enumerated by plate count method. Eighty chicken carcasses were rinsed with 400 mL of buffered peptone water with gentle shaking for 1 min. A 25-mL aliquot of the rinsate was combined with 25 mL of double-strength Bolton broth with or without rifampicin prior to microaerobic incubation at 42°C for 48 h. A loopful of each broth was streaked onto modified charcoal-cefoperazone-deoxycholate agar. Suspected colonies were confirmed by colony PCR.

Results: The comparisons of the cell counts of the five *Campylobacter* strains grown in the Bolton broth with (8.73±0.38 Log CFU/ml) or without 20 µg/mL of rifampicin (8.66±0.60 Log CFU/ml) indicated no statistical difference (P<0.05). The isolation rate of *Campylobacter* spp. was significantly higher (P<0.05) in the modified broth (44/58) than in the normal broth (0/58). Furthermore, the number of agar plates with non-*Campylobacter* spp. was much lower (P<0.05) after enrichment in the modified broth (4/58) than in the normal broth (58/58).

Significance: Rifampicin-supplemented Bolton broth is a useful option for increased recovery and reduced contamination during the isolation of *Campylobacter* spp. from chicken products.

P2-205 Comparison of Conventional Culture, Filtration, Real-Time PCR, and Digital Droplet PCR Methods for the Isolation of *Campylobacter* spp. in Fresh Produce

JUNG-WHAN CHON[1], Ji Young Jung[2], Kidon Sung[1] and Saeed Khan[1]
[1]U.S. Food and Drug Administration/NCTR, Jefferson, AR, [2]NCTR FDA, Jefferson, AR

Introduction: There have been incidents of *Campylobacter* contamination of produce. Although the incidence rate is low compared to contamination in poultry products, it is important to evaluate detection methods for *Campylobacter* on a produce matrix because of its unheated process, low infectious dose, and cross-contamination rate. However, little is known about the appropriate conventional and molecular detection methods for the pathogen in produce.

Purpose: We compared a culture method with or without membrane filtration. Concurrently, real-time PCR and digital-droplet PCR (ddPCR) were evaluated as alternative screening tools for detecting the presence of *Campylobacter* in the same samples.

Methods: Alfalfa sprout, clover sprout, coleslaw, and lettuce were spiked with the appropriate CFU of *Campylobacter* (1, 10, 100, and 1000 CFU/50g, 5 replicates for each concentration). Each sample was enriched in 100 mL of Bolton broth, followed by inoculation onto a modified charcoal-cefoperazone-deoxycholate agar (mCCDA). For the filtration, 100-µL of enrichment culture were inoculated onto a 0.65-µm sized membrane filter on the mCCDA plate. In parallel, real-time PCR and ddPCR were performed with enriched samples (1 ml each) using a primer-set targeting the *hipO* gene.

Results: There was no significant difference (P>0.05) in the number of positives among the four detection methods in coleslaw (culture method, 15/20; filter method, 18/20; real-time PCR, 19/20; ddPCR, 19/20) and lettuce (culture method, 19/20; filter method, 16/20; real-time PCR, 19/20; ddPCR, 19/20). However, in sprout samples, the detection ability of the culture method (alfalfa sprout, 1/20; clover sprout, 4/20) was significantly (P<0.05) lower than real-time PCR (alfalfa sprout, 12/20; clover sprout, 19/20), ddPCR (alfalfa sprout, 12/20; clover sprout, 19/20), or the filtration method (alfalfa sprout, 10/20; clover sprout, 18/20).

Significance: The potential risk in produce may be underestimated because of the low reliability of existing detection methods. A combination of detection methods, using approaches described in this study, should continue to be evaluated.

Poster

P2-206 Magnetic Nanoparticles-Enhanced Biosensor for the Detection of *Campylobacter* spp. in Raw Poultry Products

FUR-CHI CHEN[1] and Roger Bridgman[2]
[1]*Tennessee State University, Nashville, TN,* [2]*Auburn University, Auburn, AL*

Introduction: Rapid and accurate detection of *Campylobacter* spp. at low concentrations in raw poultry products is a critical challenge. One issue that affects many biosensor protocols is the nature of the interferences present in complex matrixes, which often results in loss of sensitivity and specificity.

Purpose: This study was to conducted to develop and validate a highly sensitive Surface Plasmon Resonance (SPR) sensor combining antibody-functionalized magnetic nanoparticles for rapid detection of *Campylobacter* in raw poultry products.

Methods: Magnetic nanoparticles, which were functionalized using monoclonal antibodies specific to *Campylobacter* spp. surface antigens, were used to separate *Campylobacter* from food matrixes. The magnetic nanoparticles used in the protocol were functioned not only as probes to selectively bind and separate *Campylobacter*, but also as an amplification regent to enhance the SPR signal. The sensor protocol was developed based on a sandwich format with paired monoclonal antibodies.

Results: *Campylobacter* was detected at concentrations as low as 100 cfu/mL and the signal showed a linear range between 1.0×10^2 and 2.4×10^5 cfu/mL. The use of magnetic nanoparticles in the SPR protocol provided three orders of magnitude in the improvement of sensitivity toward *Campylobacter* spp. compared to the regular SPR sensor with direct detection format. The specificity of the SPR sensor was examined with a cocktail of non-target bacteria (*Escherichia coli* and *Enterobacter cloacae*). No significant signal was detected in the presence of these bacteria. Of 108 raw chicken samples inoculated with a cocktail of *Campylobacter* spp. at levels between 10 and 100 cfu/g, 106 samples were detected as positive.

Significance: The developed SPR sensor has potential to provide a simple, low-cost and sensitive method for detection of *Campylobacter* spp. in poultry products.

P2-207 Same-Day Quantitative Detection of *Campylobacter* from Boot Swab Rinsates

BENJAMIN PASCAL and Adam Joelsson
Invisible Sentinel, Philadelphia, PA

Introduction: Boot swab sampling is a simple, cost-effective method for assessing *microorganism burdens* in poultry flocks prior to processing. Current rapid detection methods fail to deliver accurate, quantitative results for boot swab rinsates. Here we highlight a novel DNA-signature capture platform capable of providing same-day detection of *Campylobacter* from boot swab rinsates.

Purpose: The study evaluated the Veriflow *Campylobacter* Q assay as a tool for rapid, quantitative detection of *Campylobacter* species from boot swabs. The assay produces quantitative results in under 3 hours without sample enrichment.

Methods: 142 boot swab samples were collected from 48 locations during Fall 2016, including turkey and chicken grow houses, hatcheries and breeders. Samples were stored at 4°C until analysis. Samples were rinsed in 400 mL Buffered Peptone water, concentrated by centrifugation and recovered in assay buffer designed for poultry samples. Samples were then amplified by PCR, detected via handheld cassette, and quantified via optical reader.

Results: A positive detection threshold for the assay was set at > 300 cells/mL of rinsate. 24.6% of all samples tested were positive for *Campylobacter*. Among grow house samples, 35.6% of all turkey samples were positive (16/45) and 33.3% of chicken samples were positive (19/57). Quantitative results of positive samples show an average range of $\log_{10}$ 2.6-3.9 cells per mL of rinsate by location, identifying relative risk of downstream contamination in these flocks.

Significance: Measuring *Campylobacter* levels from boot swab rinsates can provide valuable, real-time data regarding the microbial contamination of flocks prior to being processed. This will allow for a detailed analysis of flocks prior to live-haul, enabling staging of high and low burden flocks to manage risk during production.

P2-208 Detecting *Listeria monocytogenes* in Mozzarella Cheese with the BAX® System Real-time PCR Assays for Genus *Listeria* and *L. monocytogenes* Using 24 LEB Complete Media

NISHA CORRIGAN[1], Teresa Brodeur[2], Julie Weller[1], Andrew Farnum[1], Pheakdey Ith[1] and Troy Ayers[1]
[1]*Qualicon Diagnostics, LLC, A Hygiena Company, Wilmington, DE,* [2]**, Wilmington, DE*

Introduction: *Listeria* species, including *Listeria monocytogenes*, are psychrotrophic bacterial pathogens capable of growing and multiplying under low temperatures. This characteristic has particularly impacted the dairy industry, which relies on cold temperatures to maintain product quality during extended storage. For this reason, reliable microbiological testing methods are needed to prevent *Listeria*-related illnesses associated with the consumption of contaminated milk and other dairy products.

Purpose: The purpose of this study was to assess the ability of two real-time PCR assays to detect *L. monocytogenes* in mozzarella cheese enriched in 24 LEB Complete media.

Methods: Mozzarella cheese (*n*=20) was artificially inoculated with *L. monocytogenes* to obtain fractional recovery in 125g portions and held at 4°C for 72 hours to cold stress the target organism. Samples were homogenized with 1,125 mL pre-warmed (35°C) 24 LEB Complete media and incubated at 35°C for 22-26 hours. After incubation, all samples were analyzed by real-time PCR and confirmed using the reference culture method.

Results: The real-time PCR assays for Genus *Listeria* and *L. monocytogenes* detected all 10 culture positive samples after 22 hours of enrichment in 24 LEB Complete media.

Significance: The results of this study demonstrated no significant statistical difference between the real-time PCR detection method and the reference culture method to detect *Listeria* spp. and *L. monocytogenes* in 125g of mozzarella cheese.

P2-209 Detection of Shiga Toxin-producing *Escherichia coli* in 25 Gram Samples of All-purpose Flour Using the BAX® System

Julie Weller[1], **ANDREW FARNUM**[1], Pheakdey Ith[1], Laurie Post[2], Sue Kelly[3], Thomas Donohue[3] and Holly Jaeger[3]
[1]*Qualicon Diagnostics LLC, A Hygiena Company, Wilmington, DE,* [2]*Deibel Laboratories, Long Valley, NJ,* [3]*Deibel Laboratories, Madison, WI*

Introduction: Flour is a low moisture ingredient not commonly associated with foodborne outbreaks. Although pathogens such as Shiga toxin-producing *Escherichia coli* (STEC) in raw flour are usually rendered harmless by further processing to create baked goods and other finished products, many consumers will often eat raw dough and cake batters that potentially contain these pathogenic organisms. In 2016, flour and flour-containing mixes emerged as a "new" potential carrier for pathogens after infecting 63 people with STEC.

Purpose: In response to last year's multi-state STEC outbreak, this study was designed to evaluate the BAX® System for detecting STEC in flour.

Methods: Forty 25-g samples of unbleached all-purpose flour were inoculated with a low level of *E. coli* O121 and held at room temperature for 2 weeks to acclimate the target cells. For the alternative method, twenty samples were homogenized with 225 mL of pre-warmed (42°C) mTSB+2mg/L Novobiocin and incubated at 42°C for 22 hours. Samples were tested using real-time PCR both directly from the primary enrichment and after a BHI regrowth, and all results were confirmed using the appropriate FDA-BAM procedure. For the reference method, twenty samples were enriched and plated according to the FDA-BAM method for Diarrheagenic *E. coli*.

Results: The real-time PCR assays detected 20 positive samples containing *stx*, *eae* and the O121 serogroup with 95% confirmation. The reference method resulted in 19 positives by culture.

Significance: The results of this study demonstrate that the BAX® System can detect STEC from 25g samples of flour enriched in mTSB+2mg/L novobiocin with no significant statistical difference to the reference culture method.

P2-210 Determining Whether Phages are Good Detectives of *Salmonella* Diversity Using Different Animal Production Systems in Chile as a Model

Dacil Rivera[1], Christopher Hamilton West[2], Viviana Toledo[3], Fernando Dueñas[4], Rodolfo Tardone[5], Carla Salazar[2], Luis López[6] and **ANDREA MORENO SWITT**[4]

[1]Universidad de Chile, Santiago, Chile, [2]Universidad de Chile, Facultad de Ciencias Veterinarias y Pecuarias, Santiago, Chile, [3]Universidad Andrés Bello, Santiago, Chile, [4]Universidad Andres Bello, Santiago, Chile, [5]Andres Bello University, Santiago, Chile, [6]University of Chile, Faculty of Chemical and Pharmaceutical Sciences, Santiago, Chile

❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* is a widely distributed zoonotic pathogen that can be transmitted to humans through food or by animal contact. *Salmonella* has 2,600 serovars that show distinct distribution in different animals and geographic regions; a few serovars (e.g., Enteritidis) are globally distributed. For all animal production systems, *Salmonella* is a concern, as it can enter the production systems and from there, it can reach consumers. While isolating *Salmonella* from animals is, in some cases, difficult; bacteriophages are abundant and easily isolated and characterized in the laboratory. In Chile, animal production shows considerable diversity. From backyards to industries, mostly, poultry, pigs, and cows are raised.

Purpose: The purpose of this project was to study *Salmonella* phage, using different animal production systems in Chile as models.

Methods: A total of 7 study systems (backyard poultry and pigs (*n*=35), industrial pigs (*n*=36), backyard cattle (*n*=47), industrial cattle (*n*=160), wild bird in peri-urban locations (*n*=49), wild bird – wetland (*n*=271), and backyard – South American camelids (*n*=49)) in Chile were sampled for *Salmonella* spp. and phages. These systems are located from the north to the south of Chile, including Easter Island. Isolates representing the most common serovars (Enteritidis, Typhimurium, Infantis, and Heidelberg) were used to isolate phages. The isolated phages we tested for the host range using a set of 26 isolates, representing 23 *Salmonella* serovars.

Results: We have isolated three times more phages than *Salmonella* (93 *Salmonella* versus 322 phages). The host range was found to be different in the different systems, with widest host range found in backyard poultry. Clustering of phages based on host range, showed distinct profiles according to the animal system. For example, phages from cattle prefer to lyse *Salmonella* serovars Enteritidis, Javiana, Dublin, and Agona.

Significance: This was the first study that investigated *Salmonella* phage diversity in different animal production systems.

P2-211 Genomics of *Salmonella* Obtained from Irrigation Canals in Central Chile Provides Insights in Plasmids Distribution

ANDREA MORENO SWITT[1], Joaquin Escobar[1], Viviana Toledo[2], Dacil Rivera[3], Fernando Mardones[1], Aiko Adell[1], Magaly Toro[4], Narjol Gonzalez-Escalona[5], Jorge Fernandez[6] and Maria Cristina Martinez[7]

[1]Universidad Andres Bello, Santiago, Chile, [2]Universidad Andrés Bello, Santiago, Chile, [3]Universidad de Chile, Santiago, Chile, [4]INTA, University of Chile, Santiago, Chile, [5]U.S. Food and Drug Administration, College Park, MD, [6]Instituto de Salud Pública, Santiago, Chile, [7]Instituto de Salud Publica, santiago, Chile

❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* is an important foodborne pathogen that is widely distributed in animals and in the environment. In Chile, *Salmonella* is the pathogen most commonly associated to foodborne illnesses by the Public Health Institute. The presence of *Salmonella* in irrigation canals contributes to the likelihood of contamination of fresh produce. A recent study reported the presence of multidrug resistant *Salmonella* in canals used for irrigation in Chile.

Purpose: The purpose of this study was to characterize by whole genome sequencing the genetic diversity of *Salmonella* present in irrigation canals in Chile and to identify factors related with the presence of *Salmonella*.

Methods: A total of 34 isolates of *Salmonella* were selected for whole genome sequencing with the MiSeq platform (Illumina). These isolates represent 18 different serovars isolated from irrigation canals in the Metropolitan Region, Chile. We characterized the MLST type, the presence of plasmids, prophages, and antimicrobial resistance genes. Spatial analysis was conducted in R to identify variables associated with the presence of *Salmonella* in the canals.

Results: We found a considerable genetic diversity in the sequenced *Salmonella.* Diversity was represented by 14 distinct MLST types. Our study sequenced for the first time *Salmonella* representing serovars Santiago, Corvallis, and Edinburg; which represented newly reported MLST types. We found 8 plasmid incompatibility types, including a 3kb plasmid carrying *qnrB19*, gene that encode quinolone resistance in isolates of serovars Braenderup, Heidelberg, Mbandaka, and Seftenberg. Identification of prophage content showed isolates of serovar Typhimurium containing up to 5 prophages, versus serovar Corvallis containing none. A mixed model regression analysis found an association on *Salmonella* presence and climate.

Significance: Our study indicates the importance of understanding the genetic and spatial diversity of *Salmonella* present in irrigation canals. Presence of closely related plasmids in different serovars might indicate transferable characteristics of this small plasmid.

P2-212 Reducing the Risk of *Listeria monocytogenes* in Rural Artisan Cheese in Southern Chile through Surveillance and Extension

ANDREA MORENO SWITT[1], Carla Barria[1] and Randall Singer[2]
[1]*Universidad Andres Bello, Santiago, Chile,* [2]*University of Minnesota, Saint Paul, MN*

Introduction: In southern Chile many rural families raise dairy cows, and to add value to their milk, many also produce cheese. Most of these small cheese makers lack the infrastructure and training to make safe cheese products. The cheese is typically sold to neighbors or through informal markets and thus there is no inspection of these products. The likelihood of *Listeria monocytogenes* contamination and consequent risk of listeriosis through the consumption of these artisan cheeses is unknown

Purpose: The purpose of this study was to develop a surveillance program of *Listeria monocytogenes* in artisan cheese in southern Chile.

Methods: A total of 40 small cheese producers in the Región de los Ríos, Chile, were included in the project. Each cheese location was georeferenced and a check-list was used to characterize infrastructure, processing, GMP, and SOPs. We sampled each producer four times in 2016. If *Listeria* was detected, we conducted environmental sampling and SOP training. *Listeria* strains were subtyped with PFGE to identify contamination sources and to inform corrective actions.

Results: There was considerable diversity of production practices and infrastructure among the 40 producers, and only 10 fulfill the requirements necessary to obtain a Chilean permit to produce cheese. Cleaning and sanitation, pest control, and training were identified by the producers and the check-lists as the main needs. *Listeria* was identified in 19 cheese samples from 5 producers. Identical PFGE patterns were found in food contact surfaces and in contaminated cheeses. Corrective actions controlled contamination in 3 producers. The project developed a GMP manual, a pilot plant design and an investment plan for improving the infrastructure and equipment of these small producers.

Significance: Our study has implemented a sustainable system that improves the livelihood of rural cheese producers and reduces the likelihood of *Listeria* contamination in artisan cheeses in southern Chile.

P2-213 Prevalence and Antibiotics Resistance of *Listeria monocytogenes* Isolated from Raw and Traditionally Processed Cow Milk in Ghana

JAMES OWUSU-KWARTENG[1], Alhassan Wuni[2], Fortune Akabanda[1] and Lene Jespersen[3]
[1]*University for Development Studies, Navrongo, Ghana,* [2]*University for Development Studies, Tamale, Ghana,* [3]*University of Copenhagen, Copenhagen, Denmark*

Introduction: Cow milk is processed into various traditional food products in Ghana. Currently, there is little information on the presence of *Listeria monocytogenes* in milk products in Ghana. *Listeria* poses serious public health problems and, therefore, there is a need to assess their prevalence and antibiotic susceptibility.

Purpose: This study determined the prevalence of *Listeria* spp. and *L. monocytogenes* in raw and processed cow milk sold in northern Ghana and assessed the resistance of isolated *L. monocytogenes* to selected antibiotics.

Methods: A total of 326 samples comprised of 114 raw cow milk, 56 boiled milk, 50 *nunu* (soured milk), 34 yoghurt, 54 *woagashie* (West-African soft cheese), and 18 hard/fried cheese were collected from dairy farms and market vendors for the isolation of *Listeria* spp. and *L. monocytogenes*. Isolates were characterized by biochemical tests and identified by PCR-techniques based on presence of virulence-associated genes (*plcA*, *actA*, *hlyA*, *iap,* and *prfA*). *Listeria monocytogenes* were further evaluated for resistance to ten antibiotics using the microdilution method.

Results: *Listeria* spp. was detected in 44 of 326 (13.5%) samples, of which 22 (6.7%) were positive for *L. monocytogenes*. Soft cheese had the highest prevalence (18.5%) of *L. monocytogenes* followed by raw milk (6.9%) and spontaneously fermented milk (4.0%). All *L. monocytogenes* were positive for *plcA*, *prfA*, *actA*, *hlyA*, and *iap* genes. In general, *L. monocytogenes* were susceptible to ampicillin (100%), gentamicin (100%), vancomycin (100%), streptomycin (98%), ciprofloxacin (96%) kanamycin (95), doxycycline (93%), and erythromycin (90%). However, 61% and 23% of *L. monocytogenes* isolates were resistant to neomycin and tetracycline, respectively.

Significance: This report on the presence of *Listeria* in raw and processed milk products in Ghana indicated the potential risk of listeriosis associated with the consumption of these products and, therefore, indicated the need to implement safety plans aimed at controlling *Listeria* in these products.

P2-214 Microbial Contamination Levels of Milk and Cheese Produced in Two Korean Small-scale Dairy Farms

Il-Byeong Kang[1], Dong-Hyeon Kim[1], Hong-Seok Kim[1], Dana Jeong[1], Joo-Yean Lee[2] and **KUN-HO SEO**[1]
[1]*Konkuk University, Seoul, South Korea,* [2]*Korea Livestock Products HACCP Accreditation Service, Gyeonggi province, South Korea*

Introduction: In order to produce farmstead cheeses, raw milk must be passed through various manufacturing steps, including milking, pasteurization, ripening, and storage. Control measures and hygiene practices for reducing microbial contamination at each step are essential for the production of farmstead cheeses that are safe to consume.

Purpose: The aim of this study was to investigate the microbiological quality and presence of target pathogens in teat skin, milk, and cheese and to examine the effect of farm practices on bacterial counts in a small-scale dairy in Korea.

Methods: A total of 80 swab (40 teat skin and 40 utensils) and 80 dairy product (40 milk and 40 cheeses) samples were collected from two dairy farms and examined to determine numbers of mesophilic aerobes (MA), total coliforms (TC), and *Escherichia coli* (EC), as well as the prevalence of foodborne pathogens.

Results: Teat washing and pasteurization significantly reduced levels of MA, TC, and EC, as well as the prevalences of *Staphylococcus aureus* and *Enterococcus faecalis* ($P < 0.05$). However, the prevalence of *S. aureus* and *E. faecium* was increased during the production process for farmstead cheese. In addition, *S. aureus* isolated from cheese showed a different antibiotic-resistant pattern from other isolates, suggesting post-pasteurization contamination. High levels of resistance to erythromycin and tetracycline were observed in *Enterococcus* spp. isolates.

Significance: In conclusion, teat washing and raw milk pasteurization are important processes for reducing microbial contamination in farmstead cheeses. Intervention methods for preventing post-pasteurization contamination, however, should be established to ensure the safety of the final product from cheese-manufacturing dairy farms.

P2-215 Population Dynamics of *Listeria monocytogenes* during Gouda Cheese Manufacture Using Artificially Inoculated, Unpasteurized Milk

JOELLE K. SALAZAR[1], Sartaj S. Narula[2], Christina K. Carstens[3], Arlette Shazer[1], Kristin M. Schill[4] and Mary Lou Tortorello[1]
[1]U. S. Food and Drug Administration, Bedford Park, IL, [2]IIT Institute for Food Safety and Health (IFSH), Bedford Park, IL, [3]U.S. Food and Drug Administration, Bedford Park, IL, [4]U.S. Food and Drug Administration, Division of Food Processing Science and Technology, Bedford Park, IL

Introduction: Unpasteurized milk poses a food safety concern due to possible contamination by pathogens, including *Listeria monocytogenes*. Contaminated milk used for cheesemaking has been shown to harbor this pathogen during manufacture and 60 d aging process. The FDA 2014-2016 sampling assignment revealed the presence of *L. monocytogenes* in raw milk cheeses, especially semi-soft cheeses such as Gouda.

Purpose: This study intended to determine the differences in population dynamics of *L. monocytogenes* during Gouda cheese manufacture depending on the initial inoculation levels of one or three log CFU/mL.

Methods: Unpasteurized milk was inoculated with one or three log CFU/mL of a three strain cocktail of *L. monocytogenes.* Milk was heated to 30°C prior to starter culture addition, ripening occurred for 30 min, then rennet was added. After 30 min, the curd was cut, stirred for 20 min, and 1/3 of whey volume was drained. After cooking the curd at 37°C for 20 min, it was molded and pressed overnight. Brining was conducted according to the resulting cheese weight preceding storage at 10°C. Cheese was sampled for plate count assay throughout cheesemaking and 24 h post-brine. Data were computed as log CFU/g or /mL and statistically analyzed by ANOVA, $P \leq 0.05$.

Results: No significant differences in *L. monocytogenes* populations were observed during initial manufacturing steps. After curd cutting, *L. monocytogenes* was significantly more concentrated in the curd (1.38±0.19 and 2.76±0.13 CFU/g for the 1 or 3 log CFU/mL conditions, respectively). After storage at 10°C for 24 h, the *L. monocytogenes* population increased significantly to 1.85±0.30 CFU/g in the 1 log CFU/mL condition. A significant population increase (6.82±0.03 CFU/g) was also observed after storage for the three log CFU/ml condition.

Significance: The results of the study can aid in the risk assessment of Gouda cheese processing based on initial concentrations of *L. monocytogenes* in milk.

P2-216 The Role of Inter-strain Interactions on the Growth of Matrix-Adapted and Non-Adapted *L. monocytogenes* Strains on Different Types of Cheeses

MARIA GKEREKOU, Maria Georgoulia, Anastasia Kapetanakou, Eleftherios Drosinos and Panagiotis Skandamis
Agricultural University of Athens, Athens, Greece

❖ ***Developing Scientist Competitor***

Introduction: The coexistence of different *L. monocytogenes* strains in the same food sample might influence their growth capacity and thus, the final levels at the time of consumption.

Purpose: To investigate the inter-strain interactions on the growth of matrix-adapted and non-adapted *L. monocytogenes* on Ricotta and Camembert cheeses.

Methods: Ricotta (10g) and Camembert (6x2 cm^2 pieces) cheeses were inoculated at approximately 2.5 log CFU/g or cm^2 with four matrix-adapted and non-adapted *L. monocytogenes* strains (serotypes 1/2a, 1/2b, 4b), in single and two-strain cultures (1:1 strain ratio). Strains were selected as resistant to different antibiotics for their selective enumeration on TSA-YE+antibiotics. Adaptation of cells was performed in cheese broth (1:1 cheese in Maximum Recovery Diluent) for 48h at 7°C, before inoculation of cheeses. Adaptation aimed to evaluate exclusively the inter-strain interactions, eliminating the potential impact of cheese microenvironment on strain-to-strain interactions. Cheeses were stored aerobically at 7°C (n=2x2).

Results: Adaptation of *L. monocytogenes* strains on Ricotta and Camembert resulted in similar ($p \geq 0.05$) growth responses. Both single and co-cultured adapted strains, had shorter lag phase than the non-adapted ones. Particularly, adapted strain C5 showed growth of 1.5 log CFU/g or cm^2 on the 2nd day of storage, compared to the non-adapted, which entered the exponential phase on day 4. Differences (P<0.05) in growth kinetics of some strains were observed when grown singly compared to the same strain in co-culture. Adapted and non-adapted singly cultured strain 6179 increased to 7.2 log CFU/g, whereas in co-culture remained at 5.6 log CFU/g, suggesting growth inhibition (P<0.05), when its competitor reached the maximum growth (7.9 log CFU/g). Matrix-adaptation enhanced the impact of inter-strain interactions resulting in increased differences, regarding growth kinetics, between singly and co-cultured grown strains.

Significance: The results reveal how cheese matrix determines the outcome of inter-strain interactions and therefore could assist in explaining the dominance of certain serotypes in foods of safety concern for *L. monocytogenes*.

P2-217 New Bioluminescent Alkaline Phosphatase Test for Verification of Milk Pasteurization

Delia Calderon, Paul Meighan and **NICOLE FAMILIARI**
Hygiena, Camarillo, CA

Introduction: This study introduces a new technology from Hygiena for the monitoring of Alkaline Phosphatase verification from pasteurised milk samples. The technology uses new bioluminescent substrates in a simple self-contained device using a new luminometer to measure and interpret results from samples

Purpose: To demonstrate the performance of ZymoSnap ALP as a rapid and efficient method for pasteurization verification.

Methods: Pasteurized milk from 10 supermarkets across USA covering all fat contents from 4% down to 0.1% were evaluated in 2 assays for various Hygiena luminometers. Both assays produce equivalent results to the gold standard Fluorophos system. Each milk sample was spiked with 4 levels of bovine alkaline phosphatase to the following levels 0 mU/L, 100mU/L 350mU/L and 1000mU/L. Each milk was assayed using 5 replicates with means compared and charted for correlation.

Results: The assay demonstrated excellent linearity at all milk fat contents and demonstrated an inversely proportional relationship between fat content and light output. At 4%, 2%, 1% and 0.1% the conversion of mU/L alkaline phosphatase to RLU (relative light unit) was as follows – 4% RLU= 3xmU/L, at 2% RLU=1.75mU/L, at 1% RLU=1.5mU/L and at 0.1% RLU=1.0mU/L. The correlation coefficients for the 10 milk types at n=5 was as follows 4% R^2=09561, at 2% R^2=09561, at 1% R^2=09561 and at 0.1% R^2=09561. Other flavoured milks were also tested and followed a similar trend with fat content of 4% having the largest influence on signal output and not color of milk. Strawberry milk had a conversion to RLU of 1.2 and chocolate milk a conversion of 1.1 RLU per mU/L.

Significance: The easy-to-use assay gives dairy processors of all sizes the ability to run a quick and inexpensive alkaline phosphatase assay on milks of any fat content or flavorsfor rapid pasteurisation verification.

Poster

P2-218 Bactericidal Effect of Fermented Milk with *Cudrania Tricuspidata* leaf Extract and *Lactobacillus Gasseri* Strains

SOOMIN LEE[1], Nam Su Oh[2], Kyunga Jang[1] and Yohan Yoon[1]
[1]*Sookmyung Women's University, Seoul, South Korea,* [2]*Seoul Dairy Cooperative, Ansan, South Korea*

❖ ***Developing Scientist Competitor***

Introduction: *Cudrania tricuspidata* (CT) is a traditional herb used for remedy, and it is reported as a good source of prebiotic for fermented milk. However, the antibacterial activity of the fermented milk with CT has not been studied.

Purpose: This study investigated the antibacterial activity of fermented milk with CT leaf extract and *Lactobacillus gasseri* strains.

Methods: Milks without (fermented milk) and with powdered CT leaf extracts (0.2% [wt/wt]; CT-fermented milk) were inoculated with *Lb. gasseri* strains 505, 545, 559, and 575 (ca.10^7 CFU/ml), followed by incubation at 41°C for 40 h. Minimum bactericidal concentration (MBC) of CT-fermented milk on *Listeria monocytogenes* were determined by challenging *L. monocytogenes* strains to fermented milk and CT-fermented milk (0.32 to 10 mg/ml). To investigate the bactericidal effect of CT-fermented milk in gastrointestinal tract, 12 C57BL6/N mice were treated with a combination of 100 µl fermented milk with 100 µl *L. monocytogenes* and a combination of CT-fermented milk with 100 µl *L. monocytogenes* daily for a week. Feces were plated on CHROMagar™*Listeria* on day 0, 3, 5 and 7. The mice were then euthanized, and ceca were plated on CHROMagar™*Listeria*.

Results: *L. monocytogenes* was inhibited by CT-fermented milk with *Lb. gasseri* strains 505, 559, and 575. MBCs of CT-fermented milk with *Lb. gasseri* strains 505, 559, and 575 were 5, 0.32, and 2.5 mg/ml, respectively, while *L. monocytogenes* was not inhibited by fermented milk. *L. monocytogenes* counts in feces and ceca of the mice treated CT-fermented milk were not significantly different from those of fermented milk.

Significance: The results indicate that CT may serve as an effective prebiotic in fermented milk by *Lb. gasseri*, which can improve food safety of dairy products.

P2-219 Acidification Treatments for the Control of *Listeria monocytogenes* in Model Cheese Brines

Stephanie Barnes[1], **NATHALIA MILLAN-BORRERO**[2], Jeffrey Carbonella[2], Anthony Micheletti[2] and Dennis D'Amico[1]
[1]*University of Connecticut, Department of Animal Science, Storrs, CT,* [2]*University of Connecticut, Department of Molecular and Cell Biology, Storrs, CT*

✦ ***Undergraduate Student Award Competitor***

Introduction: Immersing cheese in brine is a common method of incorporating salt to enhance flavor and control microbial growth. *Listeria monocytogenes* (*Lm*) can survive for prolonged periods in brines, posing a cross-contamination risk.

Purpose: The objective of this study was to develop and determine the efficacy of acidification treatments for reducing *Lm* counts in brines with (W) and without (NW) whey (2%).

Methods: Model brines formulated with ~25% NaCl and ~0.25% $CaCl_2$ were adjusted to pH 5.2 with lactic acid (LA). Cultures of salt and acid adapted *Lm* (10^6 CFU/mL) were allowed to acclimate in model brines for one week at 12°C prior to treatments. Brine pH was then adjusted to pH 2 with either lactic, hydrochloric (HCl), acetic (AA), or citric (CA) acid and held at room temperature for up to 360min. Subsamples were removed and neutralized (NaOH) for enumeration every 15min for 120min, and every 30min thereafter for enrichment.

Results: Overall, counts did not change during the acclimation period. Differences in *Lm* counts were observed over time between NW and W brines when acidified with CA, HCl, or LA ($P \leq 0.002$). Treatment of NW brines with HCl, AA, and LA resulted in rapid reduction in counts to below the detection limit (10 CFU/mL) within 60-90min, whereas treatment with CA took significantly longer (120min; $P \leq 0.002$). Acidification of W brines with AA and LA reduced counts to below the detection limit more rapidly when compared to other acids (105min; $P \leq 0.017$). After 90min, *Lm* were not detectable by enrichment in NW-AA and after 2h in W-AA, NW-HCl, and NW-LA brines. In contrast, HCl-W was the least effective with ~1 log CFU/mL remaining after 120min and detectable *Lm* after 360min by enrichment.

Significance: Acidification of brines presents a cost-effective and actionable approach for the reduction of *Lm* in brines as a promising preventive control.

P2-220 Screening for Genetically Modified Plants (GMO) and Identification of Non-marker Events in Food and Feed

Hans-Henno Doerries, Stefanie Wendrich, **OLAF DEGEN**, Dr. Ivo Meier-Wiedenbach, Cordt Groenewald and Kornelia Berghof-Jager
BIOTECON Diagnostics, Potsdam, Germany

Introduction: Screening for the transgenic regulatory elements P-35S and T-NOS in food and feed samples has been the standard for testing presence or absence of genetic modified (GM) plants. Meanwhile, several GM plants have been designed, that do neither contain P-35S nor T-NOS, outdating this approach. BIOTECON Diagnostics' new GMO screening strategy consists of a sophisticated combination of screening and identification assays for maximum coverage about the presence of GMOs.

Purpose: BIOTECON Diagnostics has developed new real-time PCR GMO screening and identification assays, reducing time, effort and cost of analysis to a minimum.

Methods: The foodproof GMO Screening 1 and 2 LyoKits are targeting 8 different transgenic regulatory elements in total. Additionally, new three multiplex GMO Soya and Maize identification assays for the detection of events missing the commonly used regulatory sequences for screening have been developed. The foodproof Plant Detection LyoKit can be used to check for integrity of DNA and as a process control. The assays comply with ISO 21569 and the German Food Law § 64 LFGB for the detection of genetically modified DNA sequences. An internal amplification control is included.

Results: Specificity (Inclusivity/exclusivity) was verified against different modified and non-modified plants. 45 different matrices were tested successfully, including vegetable burger, soya products and fat. A new automated extraction protocol for the KingFisher Flex enables the analysis of matrices with low DNA content like soya lecithin. The assays showed to be robust enough for a sample volume variation between 20 and 30 µl. The absolute and relative limit of detection was determined.

Significance: The flexible GMO LyoKits screening and identification assays offer an easy and cost-effective approach for the analysis of genetically modified foods.

P2-221 Psychrotolerance of *Paenibacillus odorifer* is Not Related to Phylogeny

SARAH BENO[1], Hannibal Brooks[2], Renato Orsi[1], David Kent[1], Jasna Kovac[1], Kathryn Boor[1] and Martin Wiedmann[1]
[1]*Cornell University, Ithaca, NY,* [2]*Mississippi State University, Starkville, MS*

❖ ***Developing Scientist Competitor***

Introduction: Approximately 133 billion pounds of food in the United States are wasted each year, including 32% of the fluid milk supply. Much of this food waste is due to microbial spoilage, which in fluid milk is often caused by psychrotolerant spore-formers, such as *Paenibacillus* spp.

Purpose: The purpose of this study was to evaluate the genetic variation of *Paenibacillus* spp. isolates in relation to their ability to grow at cold temperatures (6°C) in dairy products.

Methods: Twenty-eight *P. odorifer* group strains isolated from fluid milk were selected based on unique *rpoB*allelic type and tested for their ability to grow at 6°C and 10°C in Skim Milk Broth (SMB) and Brain Heart Infusion (BHI) broth over 21 days. Whole genome sequence data of all 28 isolates were compared phylogenetically and based on gene presence-absence.

Results: Of the 28 selected strains, all were able to grow at 6°C and 10°C in BHI broth and at 10°C in SMB; however, only 14 grew at 6°C in SMB. Isolates clearly cluster into three phylogenetic clades and four different growth patterns, which can be described as long lag phase, short lag phase, no growth, and die off. Phylogenetic clade II had higher representation of isolates with the die-off growth pattern, and was characterized by the loss of the gene *glnQ*, encoding for glutamine transport protein, which has been previously associated with psychrotolerance of *Bacillus subtilis*. Although the loss of *glnQ* was significantly associated with clade II, the die-off growth pattern was not.

Significance: We did not find any significant associations between growth patterns and the presence/absence of individual genes. The lack of clear gene associations ultimately complicates the development of specific detection strategies for *P. odorifer* group strains that can cause fluid milk spoilage.

P2-222 Quantitative Risk Assessment for Shiga Toxin-producing *E. coli* (STEC) in Producer-Distributor Bulk Milk Sold

ELNA BUYS, Patrick Njage and Victor Ntuli
University of Pretoria, Pretoria, South Africa

Introduction: We recently reported a high prevalence of Shigatoxin producing *E. coli* (STEC) O157 and non-O157 in raw and pasteurised producer-distributor bulk milk (PDBM) in South Africa. *Escherichia coli* has evolved from clinical novelty to primary food safety and public health concern, globally.

Purpose: The purpose of this study was to carry out a quantitative risk assessment for STEC in PDBM sold in South Africa.

Methods: Taking into account prior collected prevalence data of STEC in raw and pasteurised PDBM, and information on handling, processing, transportation and consumption patterns along the PDBM chain, probabilistic exposure models with Monte Carlo simulation were developed. Hazard characterisation was based on dose-response, using Beta-Poisson model to calculate the probability of illness from STEC. Monte Carlo simulation was carried out using @Risk software. Sensitivity analysis for the assessment of the uncertainty and variability associated with the model was also carried out. Input data used in modelling was obtained from recent published and unpublished literature from South Africa.

Results: The estimated concentration of STEC in raw and pasteurised PDBM samples was 0.12 cfu/ml and 0.08 cfu/ml, respectively. The model predicted 0.2 hemolytic uremic syndrome cases for every 20,000 consumers per annum. Sensitivity analysis to assess the uncertainty and variability associated with the model revealed boiling milk before consumption and strict control of temperature during storage along the chain have significant influence on the disease incidence.

Significance: Results from this study can be used to formulate risk-based mitigation strategies and policies under the current production and marketing conditions of PDBM in South Africa. The models can be used in other risk assessments for milk produced from similar scenarios.

P2-223 Evaluation of a Commingled Raw Milk Screening Method to Detect Tetracyclines At or Below U.S. Tolerances

ROBERT SALTER, David Douglas, David Legg, Janine Schwartz and Ryan Sullivan
Charm Sciences, Inc., Lawrence, MA

Introduction: Testing milk for non-β-lactam antibiotics before dairy acceptance is developing US/NCIMS dairy industry regulation. Tetracyclines are targeted in a pilot program. Approved methods to detect at US Tolerance, 300 parts per billion (ppb), are needed. Evaluation of methods follows FDA Center for Veterinary Medicine (FDA-CVM) accelerated tetracycline protocol.

Purpose: Evaluate tetracycline test method with dilution confirmation according to FDA-CVM criteria.

Methods: Rapid One Step Assay (ROSA) Tetracycline (Charm Sciences, Inc.) method used at CODEX cumulative tetracycline maximum residue levels (100 ppb) was adapted with a tetracycline dilution buffer to confirm US tolerance (300 ppb) without being overly sensitive (<150 ppb chlortetracycline [CTC], < 120 ppb oxytetracycline [OTC], < 70 ppb tetracycline [TC]). The adapted ROSA Tetracycline-SL (Dilution Confirmation) method was evaluated by Q-laboratories, Cincinnati, OH for 90% detection with 95% confidence using *n*=30 replicates at 5 concentrations per drug and *n*=60 negative. Somatic and bacterial interference samples *n*=60 negative and *n*=30 positives were evaluated by Eurofin, DQCI Mounds View, MN. Data on chemical interferences, freeze thawing, and assay perturbations were submitted by manufacturer. Proficiency study using 16 NCIMS laboratories was performed with 2 zero samples, 2 samples each at 300 ppb CTC or OTC and 1 sample each at 100 ppb CTC or OTC.

Results: Q-laboratory detection CTC=289 ppb, OTC=294 ppb, and TC=126 ppb compared to manufacture data CTC= 205 ppb, OTC=181 ppb, and TC= 81 ppb. There were no interferences from somatic cells (1.1 M/mL) or bacteria (>300,000 CFU/mL) or from animal drugs (36 non-tetracycline drugs at 100 ppb) or from other assay perturbations. Sixteen laboratories successfully performed the method to detect 300 ppb CTC and OTC, 64 positives of 64 replicates, while not being overly sensitive to 100 ppb CTC, 88% positive, or 100 ppb OTC, 50% positive.

Significance: The ROSA Tetracycline-SL (Dilution Confirmation) method for detecting tetracyclines in commingled milk at US Tolerances meets FDA-CVM and NCIMS criteria for acceptance.

P2-224 Thermal Inactivation of *Staphylococcus aureus* in Liquid Whey

KORI SCHERER, Brandon Wanless and Kathleen Glass
University of Wisconsin-Madison, Madison, WI

Introduction: Whey is a useful byproduct from cheesemaking. In some production facilities, the whey is transferred to large storage tanks before further processing. The excessive handling predisposes the whey to recontamination by *Staphylococcus aureus*, which produces a heat-stable enterotoxin if the whey was not sufficiently cooled. However, additional heat treatment or use of hydrogen peroxide to control pathogen growth may result in lower quality whey products. Therefore, data is needed to determine the minimum temperature and time required for pasteurization and safety.

Purpose: The objective of this study was to determine the *D*- and *z*-values of *Staphylococcus aureus* in whey.

Methods: Six lots of whey (three lots each from cheese made with or without mesophilic starter culture) were standardized to pH 6.5+0.1 with NaOH and inoculated with a three-strain mixture of *S. aureus* to yield nine log CFU/ml. Samples (1ml aliquots) were vacuum-sealed in moisture-impermeable pouches and heated to 60.0, 62.8, 65.6, or 68.3°C (140, 145, 150, or 155°F) by submersion in a water bath. For each treatment, duplicate samples were removed from heating at appropriate time intervals and enumerated for surviving *S. aureus*by plating on Baird-Parker agar overlaid with Tryptic Soy agar to aid in the recovery of heat-injured cells.

Results: There was no statistical differences in thermal inactivation rates for whey, regardless of whether it was produced with starter culture (P>0.05). Pooled data was used to calculate thermal inactivation rates. *D*-values were 1.32, 0.38, 0.12, and 0.07 minutes for samples heated at 60.0, 62.8, 65.6, or 68.3°C, respectively. The *z*-value was calculated to be 6.1°C (11.56°F) with a best-fit reference temperature of 62.8°C.

Significance: These data can be used by the dairy industry to develop pasteurization procedures to kill vegetative pathogens such as *S. aureus.*

P2-225 Inhibition of *Staphylococcus aureus* in Whey Treated with Hydrogen Peroxide during Extended Non-refrigerated Storage

BRANDON WANLESS and Kathleen Glass
University of Wisconsin-Madison, Madison, WI

Introduction: During production of cheese, liquid whey is drained from warm cheese curd at temperatures ranging between 38 and 49°C and then transferred to large storage tanks before further processing. Depending on cooling capabilities, the whey temperature may exceed 7°C for >4 hours, particularly during summer months. This environment provides conditions under which *Staphylococcus aureus* may produce heat-stable enterotoxin. Hydrogen peroxide can be added to the liquid whey to inhibit microbial growth.

Purpose: To assess the ability of whey treated with hydrogen peroxide to inhibit the growth of *S. aureus* when stored non-refrigerated for 24 hours.

Methods: Two whey types (with and without starter culture; four lots each) were inoculated with 3-log *S. aureus* per ml. Whey was then treated with 0, 10, or 100 ppm H_2O_2, and samples incubated at either 21 or 32°C. Duplicate samples were assayed for *S. aureus* at 0, 4, 8, 12, and 24 hours by plating on Baird-Parker agar.

Results: When stored at 21°C, the no-starter culture whey without H_2O_2 supported >1 log increase of *S. aureus* at 8 hours, whereas none of the other treatments supported growth for the 24 hour holding period. When stored at 32°C, the no-starter whey with 0 or 10 ppm H_2O_2 supported >1 log growth at 4 and 8 h, respectively. In contrast, no growth was observed in the 100-ppm treatment for no-starter whey or in any of the whey with starter culture. The pH of whey without H_2O_2 decreased from 6.6 to <5.0, regardless of inclusion of starter culture whereas the pH of treatments with 100 ppm H_2O_2 remained stable.

Significance: Data suggest that whey produced with a starter culture will inhibit *S. aureus* if stored at <32°C for up to 24 hours. Alternatively, *S. aureus* can be inhibited in whey through temperature-time control or addition of 100-ppm hydrogen peroxide.

P2-226 Revolutionary Screening of Residues in Raw Milk Using the Infiniplex for Milk Biochip Array Kit

J. Mahoney[1], E. Daeseleire[2], W. Reybroeck[2], L. Sibanda[1], **M. WALSLEBEN**[1], J. Porter[1], R.I. McConnell[1] and S.P. FitzGerald[1]
[1]Randox Food Diagnostics, Crumlin, United Kingdom, [2]Institute for Agriculture Fisheries and Food (ILVO), Melle, Belgium

Introduction: Comprehensive detection of veterinary drugs in milk, including all legislated antibiotics at or below relevant regulatory requirements, is important for consumer protection. Based on biochip array technology, the Infiniplex for Milk (IPM) biochip array allows simultaneous screening of 129 drug residues including mycotoxins, growth promoters, corticosteroids, anti-inflammatories and antibiotics per milk sample.

Purpose: The purpose of this study was to demonstrate the robustness of the IPM array kit in the multiplex screening of Raw Farm Milk (RFM) and Raw Tanker Milk (RTM) samples followed by LC-MS/MS confirmation (at ILVO).

Methods: Simultaneous competitive chemiluminescent immunoassays defining discrete test sites on the biochip surface were employed and applied to the Evidence Investigator analyser. Up to 48 samples can be analysed within 2.5 hours. 23 RFM and 153 RTM samples were screened followed by LC-MS/MS confirmation. Neat milk samples (25µl) were added directly to the biochips.

The LC system consisted of an Aquity UPLC. Separation was achieved on a Kinetex C18 2.1 ɔ100mm, 1.7µm column, injection volume 5µl, flow rate, 0.4 ml/min of H_2O/ACN (95/5%) + 0.3% AA. The MS equipment consisted of a Xevo TQ-MS (Waters). Analytes were determined with tandem electrospray positive or negative MS with two transitions. An Obelisc R (Sielc) 2.1 × 100mm, 5µm column was used for aminoglycosides separation. The column was held at 40°C, injection volume 10µl, eluent flow: 0.5 ml/min. The elution was performed gradually with changing amounts of H_2O + 1% FA and ACN + 1%FA.

Results: 9 (34.6%) RFM and 2 (1.3%) RTM samples screened positive on IPM biochip array for at least one drug residue, including gentamicin, kanamycin, spectinomycin, cefquinome, cephalexin and florfenicol. Samples were confirmed by LC-MS/MS (except for florfenicol as it required confirmation by an ISO17025 accredited laboratory).

Significance: These findings indicate that the use of the IPM as a multi-analytical tool revolutionises the screening capacity for drug residues in test settings, which is beneficial for consumer protection.

P2-227 Modelling the Effect of Acid and Salt Stress on the Survival and Diversity of *Listeria monocytogenes* in a Lactic Soft Cheese Stored at 4°C

THULANI SIBANDA[1] and Elna Buys[2]

[1]*Universityof Pretoria, Pretoria, South Africa,* [2]*University of Pretoria, Pretoria, South Africa*

❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* is the causative agent of the human listeriosis. As a ubiquitous organism, it is widely present in food processing plants where it causes product contamination. Continued stress exposure results in the selection of stress resistant variants with enhanced survival. Among dairy products, soft cheeses are leading causes of listeriosis outbreaks.

Purpose: The purpose of the study was to model the survival response of *L. monocytogenes* strains in a lactic soft cheese stored at 4°C after exposure to acid and salt stress and to evaluate the effect of lactate and diacetate salts on the inactivation kinetics.

Methods: *Listeria monocytogenes* strains T69, 159/10, 243 and ATCC19115 were subjected to acid and NaCl stress, then inoculated into soft cheese and stored at 4°C for 15 days. Survival data was fitted into four primary inactivation models. A secondary second order polynomial function was used to model the effect of sodium lactate and diacetate on the rate parameter of the Weibull model. GTG_5REP PCR fingerprinting was used to assess diversity of survivors.

Results: Stress treated cell inactivation was described by non-linear models ($R^2 > 0.90$). When unstressed, inactivation was best described by a convex double Weibull model ($R^2 = 0.86$). Stressed cells had significantly reduced ($P < 0.05$) inactivation rates. Addition of sodium lactate and diacetate (0.5 – 2.5% (m/v)) to the soft cheese resulted in an increase in the rate parameter of the Weibull model described by a second order polynomial function ($R^2 = 0.84$). Strain 159/10 remained the dominant strain out of 40 isolates representing survivors of acid and salt treated *L. monocytogenes* after 15 days.

Significance: Stressed *L. monocytogenes* have enhanced survival in acid soft cheese. Lactate and diacetate salts are not effective in controlling *L. monocytogenes* in lactic soft cheese. Recent food isolates survive better than laboratory strains in contaminated foods and could be more useful in predicting *L. monocytogenes* survival response.

P2-228 Growth Assessment of *Listeria monocytogenes* in Indian Cottage Cheese (Paneer) under Homemade and Industrial Scenario

VARALAKSHMI SUDAGAR[1], Sarah Leysen[1], An Vermeulen[2], Frank Devlieghere[1] and Mieke Uyttendaele[1]

[1]*Ghent University, Ghent, Belgium,* [2]*Ghent University, Gent, Belgium*

❖ ***Developing Scientist Competitor***

Introduction: Fresh cheese is an at risk product for *Listeria monocytogenes* and there have been several multistate listeriosis outbreaks and recalls reported in USA and Australia.

Purpose: No data are available on the growth assessment of *L. monocytogenes* in paneer and the safety of the final product. The goal of this study was to see if paneer acts as a vehicle that supports the growth of *L. monocytogenes* by conducting the challenge testing experiments in paneer under homemade and industrial scenarios and to compare the results with existing predictive models for validation.

Methods: Growth of *L. monocytogenes* was assessed by conducting challenge testing with an initial concentration of ca. 500 cfu/g. The paneer was prepared in the laboratory simulating homemade preparation and storage with three different pathogen contamination modes: during coagulum preparation stage, during immersion of the coagulated milk in water, and post-processing surface contamination. For the industrial scenario, using different concentrations of preservatives (2700 ppm and 1350 ppm of potassium sorbate), vacuum packaging without preservatives, and post-vacuum packaging pasteurization treatment. Each trial was conducted using three batches with two replicates each. Results of challenge testing were compared with Combase and FSSP prediction softwares.

Results: The models predicted the growth of *L. monocytogenes* when contamination occurred during the surface or immersion stage. However, the models were unable to predict the growth if contamination occurred during the coagulum preparation stage. For the homemade scenario, an increase of more than two log CFU *L. monocytogenes*/g was observed on day 10 when stored at 4°C. For the industrial scenario, a prolonged storage with no or restricted growth of the *L. monocytogenes* during several weeks was observed.

Significance: This work is the first growth assessment study of *L. monocytogenes* in paneer under artisanal homemade conditions and industrial conditions where a preservative system was included.

P2-229 Effects of Addition of Essential Oils from *Origanum vulgare L.* and *Rosmarinus officinalis L.* during the Manufacture of Minas Frescal Cheese on Viability of Starter Bacteria

MARCIANE MAGNANI[1], Helena Taina Diniz Silva[2] and Evandro Leite de Souza[3]

[1]*Federal University of Paraiba, Joao Pessoa, Brazil,* [2]*Federal University of Paraiba, João Pessoa, Brazil,* [3]*Federal University of Paraíba, João Pessoa, Brazil*

Introduction: The combined application of essential oils from *Origanum vulgare* L. (OVEO) and *Rosmarinus officinalis* L. (ROEO) has been cited as a strategy to control the growth of pathogenic and spoilage bacteria in cheeses. However, the effects of the addition of these oils during the manufacture of cheeses on viability of traditional starter bacteria remain unknown.

Purpose: This study evaluated the effects of the addition of combined OVEO and ROEO during the manufacture of minas frescal cheese on the viability of starter mesophilic cultures *Lactococcus* lactis subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* during refrigerate storage.

Methods: After the inoculation (0.01 g/L) of starter bacteria suspension (10^7 CFU/mL) in pasteurized milk, OEOV and OERO combined at their Fractional Inhibitory Concentration (1/16 MIC; 0.03 µl/g + 1.32 µl/g) were added to the cheese batter. Cheese manufactured from the same bath, without oils, was prepared similarly (control). The number of viable of *Lactococcus* spp. cells (log CFU/mL) in the cheese was counted at 1, 3, 6, 9, 12, 15, 18, and 21 days after storage at 8-10°C by serial dilution and plating on M17 agar base. Statistical analysis considered $P<0.05$.

Results: *Lactococcus* spp. in cheese containing OVEO and ROEO exhibited similar viable cells counts ($P>0.05$) compared to control system over time. The initial counts in cheese manufactured with or without OVEO and ROEO was approximately 10^8 CFU/g. After 9 days of refrigerate storage, and increase of approximately 3 log units was observed in viable counts of *Lactococcus* spp. in both cheese manufactured with or without OVEO and ROEO. For both cheeses, no increase in viable counts of *Lactococcus* spp. was observed during the remaining assessed times.

Significance: The results showed that the application of combined OVEO and ROEO did not affect the viability of traditional starter bacteria used to produce minas frescal cheese during refrigerate storage.

Poster

P2-230 Potential of Lactic Acid Bacteria Isolated from Tropical Fruits as Biopreservants in Minas Frescal Cheese

MARCIANE MAGNANI[1], Whyara Karoline Almeida Costa[2], Larissa Ramalho Brandão[2], Estefânia Fernandes Garcia[2], Marcos Santos Lima[3] and Evandro Leite Souza[2]

[1]Federal University of Paraiba, Joao Pessoa, Brazil, [2]Federal University of Paraiba, João Pessoa, Brazil, [3]Federal Institute of Petrolina, Petrolina, Brazil

Introduction: Lactic Acid Bacteria (LAB) produce organic acids and may antagonize *Listeria monocytogenes* growth. *Listeria monocytogenes* (Lm) is an important pathogen associated with cheese. LAB application as a biopreservant in cheese provides an attractive and economic control. Tropical fruits are source of LAB, however the potential of LAB from fruit as biopreservants in cheese remains unknown.

Purpose: This study evaluatee the potential of *Lactobacillus plantarum* (LPMA49) and *Lactobacillus paracasei* (LPMA108), isolated from barbados-cherry and soursop, respectively, to antagonize the growth Lm in Minas frescal cheese.

Methods: Cheese samples (9 g) were inoculated with 1 mL of LAB (105 CFU/mL) and/or Lm ATCC 19115 (10^5-10^3 CFU/mL). The experimental systems included: Cheese + Lm + LPMA49 or LPMA108; Cheese + LPMA49 or LPMA108); Cheese + Lm; Cheese + saline solution. Viable cells in the systems were determined at 0, 3, 5, 7, and 14 days of storage at 8-10ºC by serial dilution and plating on MRS Agar or *Listeria* Selective Agar. At the same time points, organic acids were quantified in cheese by high performance liquid chromatography. Statistical analysis considered $P<0.05$.

Results: Lm increased 3 log units in cheese after 14 days of storage, while in systems with LPMA49 or LPMA108 decreased up to 2.5 log units after 3 days, with no increase observed in further times assessed. After 5 days of storage, viable counts of Lm were $>10^2$ CFU/g in systems inoculated with 10^3 CFU/mL. LPMA49 and LPMA108 showed an increase of three log units over time in systems inoculated with Lm or uninoculated. The decrease of Lm was accompanied by an increase of organic acids in cheese over time. LPMA108 showed greater production of organic acids in cheese compared with LPMA49.

Significance: Results showed the antagonistic activity of LAB from fruit against Lm in cheese, suggesting a biopreservant potential for the strains tested.

P3-01 Fate of *Salmonella* and *Escherichia coli* O157:H7 in Cookie Dough during Storage: Comparison of Isolates from Different Origins

SHUANG WU, Shelli Laskowitz and Soohyoun Ahn

University of Florida, Gainesville, FL

❖ ***Developing Scientist Competitor***

Introduction: Cookie dough is recognized as a potential vehicle for *Escherichia coli* O157:H7 transmission after the multistate outbreak in 2009. It may also be contaminated with *Salmonella* for using egg as one of its ingredients. Although cookie dough has been associated with pathogens, consumers' behavior of consuming raw cookie dough is still frequently reported. The pathogens' survival in cookie dough during storage can cause foodborne illness and has not been fully studied.

Purpose: In this study, we compared the survival of *Salmonella* and *E. coli* O157:H7 isolates from different origins in commercial cookie dough.

Methods: Cookie dough samples were divided into six groups and inoculated with approximately 6.0 log CFU/g of *Salmonella* isolated from peanut outbreak, egg contaminate, and clinical samples, and *E. coli* O157:H7 isolated from cookie dough outbreak, salami, and environmental samples, separately. Cell count of each isolate was obtained during eight weeks of storage at 4°C.

Results: Overall, 0.84 to 1.30 log CFU/g reduction and 0.48 to 0.87 log CFU/g reduction was observed for *Salmonella* and *E. coli* O157:H7 in cookie dough during eight weeks of storage, respectively. Notably, *Salmonella* Tennessee isolate from peanut outbreak had much lower viable cell count than *Salmonella* Enteritidis isolates from eggs and clinical samples ($P<0.05$) after five days observation. Also, *E. coli* O157:H7 isolate from the cookie dough outbreak had a significantly lower number of viable cells than other *E. coli* O157:H7 isolates from salami and environmental samples ($P<0.05$) during eight weeks storage.

Significance: Our data suggests that *Salmonella* and *E. coli* O157:H7, once introduced, remain viable in cookie dough for at least two months. The survival of pathogens is affected by the origins of the isolates. Understanding the factors contributing to such phenomenon will be essential for the control of these pathogens in cookie dough.

P3-02 Validation of *Enterococcus faecium* as a *Salmonella* Surrogate in Thermal Treatment of Almond Meal

NURUL AHMAD[1], Hsieh-Chin Tsai[2], Ian Hildebrandt[1], Meijun Zhu[2], Juming Tang[2], Bradley Marks[1] and Elliot Ryser[1]

[1]Michigan State University, East Lansing, MI, [2]Washington State University, Pullman, WA

Introduction: *Salmonella*-contaminated raw almonds previously were linked to outbreaks of salmonellosis. Although different microbial reduction strategies can be used to treat almonds; their process effectiveness must be validated, typically using a nonpathogenic surrogate.

Purpose: This study compared thermal resistance of *Enterococcus faecium* and a *Salmonella* cocktail in almond meal (water activity (a_w) 0.45) at 80, 85, and 90°C and determined the reproducibility of results across two laboratories.

Methods: A batch of whole almonds (450 g) was inoculated with either *E. faecium* NRRL B-2354 or a five-strain *Salmonella* cocktail (Agona, Enteritidis PT30, Tennessee, Montevideo, Mbandaka, all linked to low-moisture foods) at ~ eight log CFU/g, conditioned to 0.45 a_w(±0.025) at 25°C, ground into almond meal, reconditioned to the target a_w and tested for homogeneity before sample dissemination. Isothermal treatments were performed by heating ~ one gram inoculated and equilibrated samples in aluminium test cells. Survivors were enumerated on designated differential media after incubation.

Results: Overall, *E. faecium* was more thermally resistant than *Salmonella* ($P< 0.05$) across all three temperatures. *Enterococcus faecium* and *Salmonella*, respectively, yielded $D_{80°C}$ of 34.01±0.41 and 26.53±0.24 min, $D_{85°C}$ of 17.34±0.29 and 13.17±0.10 min, $D_{90°C}$ of 8.55±0.13 and 7.24±0.08 min. Although the *z*-values were close, that of *E. faecium* (16.69±0.25°C) was lower ($P<0.05$) than that of *Salmonella*(17.73±0.08°C). These results were also reproducible between laboratories, with statistically similar ($P>0.05$) *z*-values for both *E. faecium* (MSU: 16.61±0.29°C; WSU: 16.68±0.40°C) and *Salmonella* (MSU: 17.95±0.28°C; WSU: 17.56±0.22°C).

Significance: Although *E. faecium* already is an accepted *Salmonella* Enteritidis PT30 surrogate for almonds, this study confirms the robustness of the surrogate for a broader cocktail of *Salmonellae* isolated from low-moisture foods, and reproducibility across laboratories.

Poster

P3-03 Evaluation of Dry Transfer in the Removal of *Salmonella* from Food Contact Surfaces

SIMAN LIU[1], Ian Hildebrandt[2], Stephen Burbick[2], Nathan Anderson[2], Elizabeth Grasso-Kelley[1] and Susanne Keller[2]
[1]*Illinois Institute of Technology, Bedford Park, IL,* [2]*U.S. Food and Drug Administration, Bedford Park, IL*

❖ ***Developing Scientist Competitor***

Introduction: Low-moisture food manufacturers frequently implement dry-sanitation practices to clean equipment. Consequently, the purge of production lines using inexpensive dry materials to remove microbial hazards, such as *Salmonella*, provides an attractive alternative to traditional wet-sanitation. However, the efficacy of such methods is in question, and methods to determine efficacy are not currently available.

Purpose: The goal of this study was to examine the efficacy of a dry-material purge in removing *Salmonella* from a variety of equipment materials.

Methods: A cocktail of five *Salmonella* serotypes was used to inoculate beads composed of seven different food contact materials (including several stainless steel and nonmetal materials) by submersion into the inoculum. Subsequent transfer rates to a wheat flour were determined by mixing three beads of each material type with one gram flour for 60 s, then removing the beads. Experiments were completed in triplicate beginning with fresh inoculum. Population values were statically compared using ANOVA.

Results: No significant differences ($P>0.05$) were found in the populations of *Salmonella* adhering to the beads, regardless of material. When transfer to flour was examined, significant differences were found between polypropylene and all types of stainless steel and Delrin; and between nylon, glass, Delrin, and two types of stainless steel ($P<0.05$). No significant differences were found between nylon, glass, Delrin, and 316 stainless steel precision ($P>0.05$).

Significance: Differences in *Salmonella* populations, recovered based on material types, indicated that *Salmonella* may adhere more tightly to plastics than to stainless steel or glass; making the latter materials more amendable to dry-material purge cleaning methods.

P3-04 Validation of Baking to Inactivate *Salmonella* in Model High-protein and High-fat Foods

WENQIAN WANG[1], Nathan Anderson[2], Shannon Pickens[3], Ian Hildebrandt[4] and Elizabeth Grasso-Kelley[3]
[1]*Institute for Food Safety and Health (IFSH), Bedford Park, IL,* [2]*U.S. Food and Drug Administration, Bedford Park, IL,* [3]*Illinois Institute of Technology, Bedford Park, IL,* [4]*Michigan State University, East Lansing, MI*

Introduction: Foods undergo changes in temperature and water activity (a_w) during forced hot air processes such as baking. Inactivation of *Salmonella* during these dynamic processes is not well understood and difficult to predict.

Purpose: This study investigated the impacts of oven relative humidity (RH) and matrix composition on the inactivation of *Salmonella* during baking.

Methods: Model high-protein and high-fat foods, formulated with wheat flour, soy protein, and soy oil, were inoculated with *Salmonella enterica* serovar Agona 447967 to a level of ~ nine log CFU/g, and mixed to form a homogenous dough. Dough samples (57 mm diameter by 6 mm thick) were baked (three samples per dwell time, six dwell times per condition) in a laboratory-scale oven at 120°C (10% RH) and 85°C (20%, 35% RH, 50% RH), respectively. Temperature and water activity (a_w) were measured at the surface and geometric center of the product during baking. Processed samples were collected in sterile bags and immediately cooled in an ice-water bath. *Salmonella* was enumerated on trypticase soy agar supplemented with yeast extract and incubated at 37°C for 24 h.

Results: Reductions of 5.12±023, 5.11±0.36, 4.55±0.31, and 4.78±0.51 log CFU/g were achieved after 40 min at 120°C/10% RH, 90 min at 85°C/20% RH, 50 min at 85°C/35% RH, 8 min at 85°C/50% RH, respectively, in the high-protein model food. Similar results were achieved in the high-fat matrix. The a_w at the geometric center of the product (initially at a_w =0.98) did not change appreciably during baking. However, a_w at the product surface, the location of least lethality, decreased significantly ($P<0.05$) during baking; the decreases were more pronounced at the lowest oven RH.

Significance: Surface drying caused by low oven RH conditions increased *Salmonella* survival in food products. Therefore, both oven temperature and RH should be monitored during baking process.

P3-05 Multilaboratory Comparison of Thermal Resistance of *Enterococcus faecium* and *Salmonella enterica* in Peanut Butter

IAN HILDEBRANDT[1], Shannon Pickens[2], Soon Kiat Lau[3], Jeyamkondan Subbiah[3], Nathan Anderson[4], Bradley Marks[1] and Elizabeth Grasso-Kelley[2]
[1]*Michigan State University, East Lansing, MI,* [2]*Illinois Institute of Technology, Bedford Park, IL,* [3]*University of Nebraska-Lincoln, Lincoln, NE,* [4]*U.S. Food and Drug Administration, Bedford Park, IL*

Poster

Introduction: *Enterococcus faecium* NRRL B2354 has been identified a nonpathogenic surrogate for *Salmonella* in almond pasteurization processes. There is a critical need to characterize and validate *E. faecium* against *Salmonella* in other low-moisture foods in order to meet the industry need for a nonpathogenic surrogate for in-plant validations. Determination of thermal resistance at multiple laboratories provides additional confidence in the thermal destruction values for use in establishing food process parameters.

Purpose: This study compared the thermal resistance of *E. faecium* and a *Salmonella enterica* cocktail in peanut butter and evaluated the reproducibility of the results obtained by two laboratories.

Methods: A five-strain *Salmonella* cocktail and *E. faecium* diluted 1:1 with peanut oil and emulsified with Tween 80 were used to inoculate commercial peanut butter. Samples were equilibrated to 25% relative humidity before dissemination to two laboratories. Aluminum test cells were filled with inoculated peanut butter in a humidity-controlled chamber and isothermally treated at three temperatures in the range 85 to 100°C, depending on the organism. After treatment, samples were serially diluted and enumerated on appropriate differential agar. Using organism survival ratios, *D*- and *z*-values were calculated using Matlab nonlinear fitting tools.

Results: The $D_{90°C}$ for *E. faecium* (21.35±0.20 min) was greater ($P\leq0.05$) than for *S. enterica* (12.62±0.15 min). Differences in *D*-values between laboratories were significant for two temperatures, but the nominal difference remained ≤12%. The *z*-value (14.94±0.34 and 15.49±0.45ºC) for *S. enterica* was similar ($P \geq 0.05$) between laboratories. While the *z*-values for *E. faecium* were significantly different between laboratories (12.97±0.14 and 12.23±0.14ºC); the actual difference was small (0.74°C).

Significance: *Enterococcus faecium* was more thermally resistant than *S. enterica* at each temperature evaluated. Overall, results were reproducible between laboratories; however, the instances of unattributable differences reiterate the importance of cross-laboratory validations.

P3-06 Inactivation of *Salmonella* spp. and Surrogate Bacteria on Cashews and Macadamia Nuts Exposed to Commercial Propylene Oxide Processing Conditions

THOMAS SAUNDERS, Monica Ponder, Robert Williams, Jian Wu, Kim Waterman and Kendall Fogler
Virginia Tech, Blacksburg, VA

❖ ***Developing Scientist Competitor***

Introduction: Illnesses and recalls due to *Salmonella* on tree nuts has prompted increased implementation of processing interventions, including propylene oxide (PPO) fumigation. There is need to validate a large number of product configurations and processing parameters warranting the identification of a surrogate that can be used in the processing facility.

Purpose: The inactivation of three potential surrogate bacteria was compared to that of *Salmonella*, after subjection to propylene oxide processing, in accordance with the United States Environmental Protection Agency label instructions.

Methods: Macadamia nuts and cashews were inoculated with a cocktail of TSA-grown *Salmonella enterica* strains and one of three potential surrogates; *Enterococcus faecium, Pediococcus acidilactici,* or *Staphylococcus carnosus*. Samples were dried, packaged in polywoven polypropylene bags, and placed within uninoculated nuts. PPO treatments were performed by Cosmed, using their proprietary process. Samples were returned for enumeration by plating onto appropriate selective media and TSA overlaid with XLT-4. Processing was completed three times, with six samples per process. The mean log CFU/g reductions of *Salmonella* and each potential surrogate, within a sample and amongst all trials, were compared using a paired T-test; $P<0.05$ was considered significant.

Results: PPO processing resulted in significant differences in the mean reduction log CFU/g of *S. enterica* (7.3±0.19) and *E. faecium* (6.4±0.31) and *P. acidilacti* (6.3±0.33) on whole macadamia nuts. On cashews the mean reduction log CFU/g of *S. enterica* (5.2±0.22) was not significantly different from that of *E. faecium* (5.2±0.25) after PPO processing, however reduction of *P. acidilacti* (4.1±0.25) was significantly different on cashews. Reduction of *S. carnosus* was greater than that of *S.enterica*.

Significance: Reductions of the target bacteria were significantly different for the two nut types, despite processing at the same time under the same parameters. This indicates the importance of comparing the efficacy of surrogate bacteria for the food of interest.

P3-07 *Enterococcus faecium* as a Surrogate for *Salmonella* in Thermal Treatment of Nonfat Milk Powder

SHUXIANG LIU[1], Nurul Ahmad[2], Jie Xu[1], Ian Hildebrandt[2], Elliot Ryser[2], Meijun Zhu[1], Bradley Marks[2] and Juming Tang[1]
[1]Washington State University, Pullman, WA, [2]Michigan State University, East Lansing, MI

❖ ***Developing Scientist Competitor***

Introduction: Nonpathogenic surrogates play an important role in evaluating the efficacy of pathogen-reduction processes for low-moisture food products. *Enterococcus faecium* NRRL B2354 is now an accepted *Salmonella* surrogate for validating almond pasteurization processes; however, very limited information is available to support its use in milk powder.

Purpose: The objectives were to: (1) compare thermal resistance of *E. faecium* and *Salmonella* in nonfat milk powder (a_w 0.25) at 85, 90, and 95°C and (2) evaluate the repeatability of the protocol by comparing results across two laboratories.

Methods: Nonfat milk powder was inoculated with either *E. faecium* or a five-strain *Salmonella* cocktail (Agona, Enteritidis PT30, Tennessee, Montevideo, Mbandaka; all linked to low-moisture foods) and equilibrated to a_w 0.25 at 23°C for three to four days at Washington State University (WSU). After vigorously shaking to eliminate caking/clumping, ten 1-g subsamples were plated to assess homogeneity of the inoculum. Half of the samples ($10^{8.0\pm0.2}$ CFU/g) were shipped to Michigan State University (MSU) for isothermal treatment in parallel with WSU. Inoculated samples (~0.7g) isothermally heated (85, 90, or 95°C) in sealed aluminum test cells were periodically removed, cooled, and plated for survivors to calculate thermal inactivation parameters (*D*- and *z*-values).

Results: Both organisms exhibited log-linear kinetics at all temperatures tested. At all temperatures, *D*-values for *E. faecium* were significantly higher ($P<0.05$) than those for *Salmonella*. *D*-values for *E. faecium/Salmonella* were $D_{85°C}$ = 48.8±3.9/13.9±0.3, $D_{90°C}$ = 2.52±0.9/6.7±0.1, and $D_{95°C}$ = 13.2±0.6/3.5±0.1 min, respectively. *Enterococcus faecium* exhibited a higher *z*-value (17.6°C) than *Salmonella*(16.6°C).

Significance: *Enterococcus faecium* appears to be a valid surrogate for assessing thermal inactivation of *Salmonella* in nonfat milk powder, based on a higher surrogate thermal resistance.

P3-08 The Effect of Process Air Velocity, Humidity, and Product Moisture on *Salmonella* Inactivation on Almonds

FRANCISCO GARCÉS-VEGA, Kaitlyn Casulli and Bradley Marks
Michigan State University, East Lansing, MI

❖ ***Developing Scientist Competitor***

Introduction: Understanding microbial inactivation is necessary to develop robust models that can be used to validate processes. Most modeling efforts in low-moisture foods have been based on data from iso-conditions or assumed that relevant factors remain constant. However, industrial processes are highly dynamic, which can significantly affect inactivation response.

Purpose: The objective was to evaluate the effects of process air velocity on the inactivation response of *Salmonella* on almonds.

Methods: Inoculated almonds with *Salmonella* Enteritidis PT30 at seven to eight log CFU/g were equilibrated to water activity (a_w) of 0.38 or 0.65 (2.8 and 6.2% moisture content, dry basis, respectively). Samples (13 g) were processed at two air velocities (i.e., high and low) and two process humidities (<3% Mv and 30% Mv), at 121°C. Dynamic product surface temperature, moisture content, process dew point, and a_w were recorded. Inactivation data were used to estimate parameters of a Bigelow-type log-linear model, via nonlinear regression of the integrated form, including terms for temperature (zT), process humidity (zM), and air velocity (aV).

Results: Models were fitted with and without the aV term. Model errors (RMSE) ranged from 0.51 to 0.62 log CFU/g. Distinct parameters were obtained for each model; the aV parameter (-0.39) indicated that air velocity had a large effect on microbial inactivation, independent of the T(t) effect. Analysis of residuals indicated there also is an effect of product moisture content ($P<0.05$), of which the best model form is still unclear.

Significance: Overall, the results suggest that air velocity impacts the resistance of *Salmonella* in thermal inactivation processes, independently of temperature effects, likely reflecting velocity effects on the relative influence of process humidity and product moisture.

Poster

P3-09 Effect of Temperature, Water Activity, and Structure on *Salmonella* Thermal Resistance in Multiple Wheat Products

RENEE SCHWARTZ, Justine Williams, Pichamon Limcharoenchat, Nicole Hall, Michael James and Bradley Marks
Michigan State University, East Lansing, MI

✦ ***Undergraduate Student Award Competitor***

Introduction: Wheat kernels, meal, and flour are low-moisture products with potential for *Salmonella* contamination. Therefore, pathogen reduction processes are needed, and must be validated, for cereal products. However, many factors affect *Salmonella* thermal resistance, such as product structure and water activity (a_w), but little is known about the effects.

Purpose: Therefore, the objective was to quantify the effects of product structure, a_w, and temperature on *Salmonella* thermal resistance on/in wheat products.

Methods: Wheat kernels were surface-inoculated with *Salmonella* Enteritidis PT30 and equilibrated in controlled-humidity chambers to 0.25, 0.45, or 0.65 a_w. After equilibration, kernels (50 g/batch) were ground (in the humidity chamber) into whole-grain meal (25 s) and flour (60 s), and then equilibrated, again. Equilibrated samples were treated isothermally (in triplicate) in vacuum-sealed plastic bags (seven kernels/bag, ~0.4 g) and aluminum test cells (flour and meal, ~0.6 g) in a water bath (80, 85, or 90°C) and then removed at eight equal time intervals, immediately cooled in an ice bath, and plated on modified trypticase soy agar. The plates were incubated for 48 h at 37°C, and then enumerated.

Results: As expected, *Salmonella* thermal resistance decreased ($P<0.05$) with increasing temperature and water activity. For example, *D*-values on kernels at 0.45 a_w were 9.3±0.5, 3.3±0.3, and 1.1±0.1 min at 80, 85, and 90°C, respectively. However, there was no effect ($P>0.05$) of product structure on thermal resistance at 0.45 a_w.

Significance: This is the first known study to report *Salmonella* thermal inactivation kinetics on an intact cereal grain. The results indicate that a_w affects the thermal resistance of *Salmonella* Enteritidis PT30, but that structure of cereal grain products may not be a significant factor in application of thermal inactivation parameters.

P3-10 Direct Comparison of the Modes of Cross-contamination Associated with *Salmonella* during Almond Processing

JOANNA CARROLL, Quincy Suehr, Philip Steinbrunner, Bradley Marks, Elliot Ryser and Sanghyup Jeong
Michigan State University, East Lansing, MI

✦ ***Undergraduate Student Award Competitor***

Introduction: Outbreaks associated with *Salmonella* in low-moisture foods, such as tree nuts, are a concern, due to lack of understanding about the factors contributing to cross-contamination at each step during processing, with different modes of transfer between kernels and shell pieces.

Purpose: This study aimed to compare different transfer modes of cross-contamination associated with *Salmonella* during almond shelling (shell to kernel) and sorting (kernel to kernel).

Methods: Almond shell pieces and kernels (200 g) were inoculated with *Salmonella* Enteritidis PT30. The inoculated kernels (5 g), shell pieces (5 g) and uninoculated kernels (200 g) were conditioned at 0.40 a_w. Uninoculated kernels and either inoculated shell pieces or kernels were then mixed in a stainless steel drum (140 mm diameter, 64 mm depth) for the total number of rotations (TNR) of 5, 20, 40, and 80 revolutions (rotational speeds of 8, 16, and 24 rpm for 10 to 300 s), in triplicate. At each condition, uninoculated samples (four kernels) were retrieved, stomached, and enumerated for *Salmonella* on modified trypticase soy agar.

Results: Initial inoculation levels on the kernels (8.37±0.50 log CFU/g) and shell (5.94±0.04 log CFU/g) were significantly different ($P<0.05$). The maximum bacterial load transferred from shells to kernels (2.36±0.26 log CFU/g) was lower ($P<0.05$) than that of kernels to kernels (4.53±0.41 log CFU/g). However, there was no significant difference in shell inoculation level (5.55±0.41 log CFU/g, $P=0.13$) before and after the transfer experiment, which indicates that most bacteria remained on the shell.

Significance: Understanding bacterial cross-contamination modes and mechanisms is critical information for secondary modeling, which will enable elucidation of the cross-contamination pathways of *Salmonella* for an entire processing system.

P3-11 Corn Oil Enhances the Ability to Detect *Salmonella* Montevideo in Spices

NICOLE ADDY[1], Laura Ewing[1], Darcy Hanes[2] and Junia Jean-Gilles Beaubrun[1]
[1]U.S. Food and Drug Administration, Laurel, MD, [2]U.S. Food and Drug Administration, CFSAN, Laurel, MD

Introduction: Phenolic compounds associated with essential oils of spices and herbs possess a variety of antioxidant and antimicrobial properties that interfere with *Salmonella* detection from fresh and dried products. Corn oil was reported to increase the survival of *Salmonella* growth during preenrichment.

Purpose: The goal of this investigation was to evaluate the effect of corn oil as an additive to the preenrichment broth used for the recovery of *Salmonella* from various spices.

Methods: The spices evaluated were Jamaican allspice, Vietnamese cinnamon, cinnamon sticks, whole cloves, whole thyme leaves, onion powder, garlic powder, ground black pepper, and Turkish oregano. Each spice was artificially contaminated with *Salmonella enterica* Montevideo (1,000 CFU/25 g) and aged for two weeks at room temperature. The aged samples were preenriched in TSB with and without 2% (v/v) corn oil. Following 18 to 24 h of incubation at 37°C, aliquots from both preenrichment broths were transferred to selective enrichment broths and subsequently plated on Xylose-Lysine-Tergitol 4 (XLT-4) Agar for enumeration.

Results: The results demonstrated a statistically significant increase ($P\leq0.008$) of *Salmonella* recovery when corn oil was added to the preenrichment broth for whole cloves and onion powder. Vietnamese cinnamon, cinnamon sticks, onion powder, and garlic powder did not show recovery of *Salmonella*, regardless of the method used. The remaining spices had *Salmonella* recovery with corn oil.

Significance: The data presented demonstrated that the recovery of *Salmonella* from some spices is enhanced by the addition of corn oil to the preenrichment broth. This will increase the FDA's ability to detect pathogens in difficult spice matrices.

P3-12 Effect of Long-term Almond Storage on Survival and Resistance of *Salmonella* to Heat and X-ray

PHILIP STEINBRUNNER, Pichamon Limcharoenchat, Bradley Marks and Sanghyup Jeong
Michigan State University, East Lansing, MI

✦ ***Undergraduate Student Award Competitor***

Introduction: The growth of *Salmonella* is naturally inhibited in low-moisture foods due to low water activity. However, *Salmonella* can survive in these foods for extended periods and pose a food safety risk. Therefore, it is important to understand survival and resistance characteristics of *Salmonella* in low-moisture foods.

Purpose: The purpose of this study was to quantify *Salmonella* survival during long-term almond storage, and the subsequent effect on thermal and x-ray resistance.

Methods: Almonds were inoculated with *Salmonella* PT30 and conditioned to 0.45 a_w. For thermal treatment, individual almonds were vacuum-packed in plastic bags, heated in an isothermal water bath (~80°C), pulled at time steps, and cooled in ice baths. For x-ray treatment, four gram samples were irradiated utilizing a 70 kV x-ray irradiator at doses targeting one- to five-log reductions. Samples for both treatments were enumerated to determine initial inoculation and D_{10}-values. The remaining almonds were stored in airtight canisters. Almonds were sampled, equilibrated to 0.45 a_w, treated, and enumerated after 0, 7, 15, 27, and 66 weeks of storage.

Results: The initial inoculum level in the almonds (8.53±0.15-log CFU/g) decreased (P<0.05) after 27 weeks (7.80±0.11-log CFU/g). However, thermal resistance of *Salmonella* was not significantly (P>0.05) different after 27 weeks ($D_{80°C}$ ~23.8±2.2 min). In contrast, the resistance to 1.31 kGy of x-ray irradiation was increased (P<0.05) from week 0 (2.97±0.342-log reductions) to week 27 (1.24±0.359-log reductions).

Significance: The population of *Salmonella* in low-moisture food decreased over six months. Although *Salmonella* thermal resistance did not change during this period, irradiation resistance increased significantly. These findings suggest mechanism-specific effects of storage on subsequent resistance to lethal treatments.

P3-13 Quantification of Adhesion Force of *Salmonella* Attached to Food Grade Surfaces in Low-moisture Environments

QUINCY SUEHR, Bradley Marks, Elliot Ryser and Sanghyup Jeong
Michigan State University, East Lansing, MI

❖ ***Developing Scientist Competitor***

Introduction: Understanding pathogen adhesion to food grade surfaces, such as #304 stainless steel, in low moisture environments is critical to model cross-contamination of *Salmonella* and to remove bacteria from surfaces.

Purpose: This study aimed to develop a method to determine the adhesion force of *Salmonella* at a large scale in a low moisture environment, and validate using individualized bacterium adhesion data.

Methods: *Salmonella* Enteritidis PT 30 cells were attached on square stainless steel coupons (#304, 5 cm^2) of different surface finishes (984 nm and 9.34 nm root mean square (RMS) roughness) in five ml of phosphate buffer solution (PBS), with gentle stirring for two hours, after which coupons were removed and desiccated overnight. Samples were then centrifuged at relative centrifugal forces (RCF) varying from 0 to 40,000×g; the higher the centrifugal force, the more bacteria detach. Bacteria remaining on the coupons were recovered and enumerated on modified trypticase soy agar.

Results: The initial *Salmonella* populations on the rough and smooth stainless steel coupons were 4.01±0.05 log CFU/cm^2 and 4.38±0.07 log CFU/cm^2, respectively, which indicating that a greater (P<0.05) number of bacteria adhered to the smoother surface. After centrifuging at 2990×g, the rough and smooth surfaces resulted in *Salmonella* populations of 3.84±0.19 log CFU/cm^2 and 3.62±0.32 log CFU/cm^2 on the surface, respectively, with the latter being reduced significantly (P<0.05). These results suggest that surface roughness has a significant effect (~10 times) on the force required to detach bacteria (approximated value of 29.3 nN compared to 3.2 nN for rough and smooth surfaces, respectively).

Significance: Quantifying the adhesion forces at the individual bacterium scale can help develop and validate first-principle based cross-contamination models.

P3-14 Is *Enterococcus faecium* an Appropriate Surrogate for *Salmonella* in Thermal Process Validation of Cocoa Powder?

HSIEH-CHIN TSAI[1], Xia Song[1], Juming Tang[1], Bradley Marks[2] and Meijun Zhu[1]
[1]Washington State University, Pullman, WA, [2]Michigan State University, East Lansing, MI

Introduction: *Salmonella* is a growing concern in low moisture foods including chocolate. Cocoa powder, an essential ingredient of chocolate, has been observed for *Salmonella* contamination since the late 1960. High temperature processing is a primary method to inactivate microbial contamination. Although *Enterococcus faecium* is an accepted surrogate of *Salmonella* for validating almond thermal processes, no published information is available to support its use in cocoa powder.

Purpose: To compare thermal resistance of *E. faecium* and a *Salmonella* cocktail in cocoa powder (0.30 water activity (a_w)) at 70, 75, and 80°C.

Methods: Natural unsweetened cocoa powder was inoculated with *E. faecium* or a three-strain *Salmonella* cocktail (*Salmonella* Enteritidis PT30, *Salmonella* Tennessee K4643, and *Salmonella* Agona) at 1×10^9 CFU/gram, conditioned to 0.3 a_w (25°C), a typical a_w of cocoa powder. The inoculated cocoa powder was then subjected to isothermal inactivation (70, 75, 80°C) in aluminum test cells (0.4 g sample). Survivors at selected treatment time points were enumerated to determine *D*- and *z*-values.

Results: Both *E. faecium* and *Salmonella* exhibited log-linear kinetics at all temperatures. The *D*-values of *E. faecium* and *Salmonella* cocktail were 105.4±4.4 and 46.0±2.3 min at 70°C, 40.7±3.3 and 22.1±0.7 min at 75°C, 18.3±0.1 and 13.3±0.1 min at 80°C, respectively. *D*-values for *E. faecium* were significantly higher (P<0.05) than those for *Salmonella* at all tested temperatures; *E. faecium* had a closer *D*-value to that of *Salmonella* at 80°C, which warrants further comparison of thermal inactivation at higher temperatures. *Z*-values for *E. faecium* NRL B-2354 and *Salmonella* cocktail were 13.1 and 18.5°C, respectively.

Significance: *Enterococcus faecium* appears to be an appropriate surrogate for assessing industrial thermal processes of cocoa powder in inactivation of *Salmonella*.

Poster

P3-15 Almond Surface Components Increase Resistance of *Salmonella* Enteritidis PT30 Under Low-moisture Environment

HSIEH-CHIN TSAI, Lina Sheng and Meijun Zhu
Washington State University, Pullman, WA

Introduction: *Salmonella* is linked to severe foodborne outbreaks related to raw almonds. *Cinnamon cassia* oil is a widely used spice with broad antimicrobial effect. However, limited information is available about its inhibitory effect against *Salmonella* strains.

Purpose: To evaluate antimicrobial effects of cinnamon oil against *Salmonella* in a media setting and on almonds.

Methods: Antimicrobial effects of cinnamon oil against *Salmonella* Enteritidis PT30 and *Salmonella* Tennessee K4643 were evaluated by disk diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and death curve. Its inhibitory effects against *Salmonella* on almond or paper disks, a carrier to mimic low-moisture environment, were further examined during storage at room temperature.

Results: The disks with 20µl of 4% (v/v) cinnamon oil resulted in inhibition zones of 1.93 and 1.63 cm for *Salmonella* Enteritidis PT30 and *Salmonella* Tennessee K4643, respectively. MIC and MBC of cinnamon oil against both strains were 0.05% (v/v) and 0.1% (v/v), respectively. Cinnamon oil at 1.5× MBC reduced both strains in LB broth under the detection level (more than a six-log reduction) within one hour. *Salmonella* established either on almond or paper disk was very stable during seven to nine weeks of storage at room temperature, there was ~ one-log reduction over storage. 0.4% Cinnamon oil treatment initially caused ~ one and 2.6-log reduction of *Salmonella* on almond and paper disk, respectively compared to PBS control. Additionally, cinnamon oil treatment resulted in ~ two-log reduction of *Salmonella* on almond during nine-week of storage, while, on paper disk, reduced *Salmonella* below the detection level after four weeks of storage.

Significance: Cinnamon oil demonstrates antimicrobial efficacy against *Salmonella*, and has the potential to be used as a promising natural compound to control *Salmonella* on almonds. Food matrix or almond skin components increased *Salmonella* resistance against cinnamon oil.

P3-16 Fate of *Listeria monocytogenes* in Cocoa Powder during Isothermal Inactivation

HSIEH-CHIN TSAI, Marizela Silva, Juming Tang and Meijun Zhu
Washington State University, Pullman, WA

Introduction: *Listeria monocytogenes* can survive in dry condition for a long period of time. Despite an increasing number of studies addressing *Salmonella* inactivation in low-moisture foods, there is a general lack of knowledge related to *L. monocytogenes* inactivation in low moisture foods during thermal processing and to the factors impacting their survival in low moisture food. Cocoa powder is an essential ingredient and widely incorporated in different disserts and drink and thus a possible source of *L. monocytogenes* contamination.

Purpose: To evaluate the thermal resistance of *L. monocytogenes* in cocoa powder and further investigate the impact of water activity (a_w) on its survival in cocoa powder.

Methods: Natural unsweetened cocoa powder (Hershey's) was inoculated with three-strain *L. monocytogenes* cocktail (~9.0 log CFU/g), equilibrated to water activity ($a_{w,\ 25°C}$) to 0.3 or 0.45, then subjected to isothermal treatments using aluminum TDT test cell containing 0.4 g of inoculated and equilibrated sample. The survivors were enumerated on TSAYE plates.

Results: Inactivation data resulted from different temperatures at both a_w showed log-linear trend which was used to obtained thermal inactivation parameters. Thermal resistance of *L. monocytogenes* at 0.3 a_w was significantly higher than that at 0.45 a_w across all three temperatures. *Listeria monocytogenes* at 0.3 a_w and 0.45 a_w had $D_{70°C}$ of 7.44±1.17 and 4.77±0.28 min, $D_{75°C}$ of 3.62±0.03 and 2.84±0.09 min, and $D_{80°C}$ of 1.35±0.04 and 1.06±0.05 min, respectively, *z*-value at 0.3 a_w and 0.45 a_w was 13.48 and 15.31°C, respectively.

Significance: *Listeria monocytogenes* demonstrated much higher thermal resistance in cocoa powder compared to the high moisture foods, which is impacted by a_w. Data provide valuable information for industry to validate thermal processing for control of *L. monocytogenes* in low moisture foods.

P3-17 Assessment of Survival and Virulence of *Salmonella* in Low-moisture Foods

VICTOR JAYEOLA[1], Jeffrey Farber[2] and Sophia Kathariou[1]
[1]North Carolina State University, Raleigh, NC, [2]University of Guelph, CRIFS, Department of Food Science, Guelph, ON, Canada

Introduction: Low-moisture foods (LMF) have been implicated in multiple outbreaks of salmonellosis. However, mechanisms mediating survival and virulence of *Salmonella* in such foods remain poorly understood

Purpose: To assess survival and virulence of *Salmonella* in low moisture foods.

Methods: A two-strain mixture of *Salmonella enterica* serotypes Typhimurium and Enteritidis was used to inoculate two model LMF, chocolate and inshell pistachios. Products (100 g) were inoculated with four ml of the *Salmonella* cell suspension, dried for one to three hours until a_w approximated that of the uninoculated product, aliquoted into 15-ml centrifuge tubes and stored in the dark at 22°C. *Salmonella* populations immediately after inoculation, after drying and at 1, 4, 6, 10, 15, and 21 days were determined in triplicate on nonselective (TSA-YE) and selective (XLD) media. Virulence was tested in the insect *Galleria mellonella* model by injecting 10 µl of rinsate from products at one day into the last left proleg of 10 larvae. Rinsate from uninoculated products were also injected as controls. The larvae were incubated at 37°C and larval mortality was daily monitored.

Results: The population of *Salmonella* in the inoculated products was eight and nine log CFU/g immediately after inoculation and 7.3 and 8.0 log CFU/g after drying for chocolate and pistachios, respectively. Populations decreased to 4.5 and 7.5 log CFU/g by 21 days in chocolate and pistachios, respectively. Recovery of *Salmonella* from either product on TSAYE and XLD was similar. Larvae inoculated with 3.4 log CFU/ml of the cocktail had a mortality of 100% after 24 h compared to 80% of larvae inoculated with 3.9 log CFU/ml cells from chocolate and 30% for larvae inoculated with 3.7 log CFU/ml cells from pistachios. The mortality of controls was 0 and 10% for uninoculated pistachios and chocolate, respectively.

Significance: Findings show that *Salmonella* can survive in chocolate liquor and pistachios and that cells adapted to the LMF environment retained virulence.

P3-18 Genetic Diversity, Antimicrobial Resistance, and Virulence Profile of *Salmonella* Isolated from the Peanut Supply Chain

Aline von Hertwig[1], Dionisio Amorim Neto[1], Monique Casas[2] and **MARISTELA DA SILVA DO NASCIMENTO**[1]
[1]University of Campinas (UNICAMP), Department of Food Technology, Faculty of Food Engineering (FEA), Campinas, Brazil, [2]Instituto Adolfo Lutz, Campinas, Brazil

Introduction: Due to recent outbreaks associated with low water activity food such as peanuts, *Salmonella* is now considered a risk for these products. Despite this, little is known about the characteristics and resistance of *Salmonella* isolated from peanuts.

Purpose: This study was conducted to identify and characterize *Salmonella* strains isolated the from peanut supply chain in Brazil.

Methods: Thirty-nine *Salmonella* strains isolated from different stages of the peanut supply chain were subtyped with pulsed-field gel electrophoresis (PFGE). These strains were evaluated for antimicrobial resistance (ampicillin, ceftazidime, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, cephaloxime, nalidixic acid, sulfonamide, streptomycin, trimethoprim, and tetracycline) using the disk diffusion assay. In addition, the presence of antimicrobial resistance genes (bla_{tem}, bla_{pse}, *aad*A1, *flo*R and *tet*(G)) and virulence genes (*inv*A, *ipf*A, *avr*A, and *agf*A)were investigated by PCR.

Results: Eight PFGE profiles were identified among six serotypes (*Salmonella* Muenster, *Salmonella* Miami, *Salmonella* Glostrup, *Salmonella* Javiana, *Salmonella* Oranienburg, *Salmonella* Yoruba) and were grouped into four clusters. Overall, a high resistance to sulfonamide (97.4%) was observed. Only one strain (2.5%) isolated from the postharvest step had resistance to sulfonamide and ampicillin. The strains had intermediary resistance to streptomycin (46.1%), kanamycin (33.3%), and ceftazidime (30.7%). The antimicrobial resistance genes related to ampicillin, cephaloxime, trimethoprim, chloramphenicol, and tetracycline were not detected in the analyzed strain. Virulence genes were found in isolates from different stages of the supply chain. *Inv*A and *agf*A genes were detected in all 39 strains, whereas the *avr*A gene was detected in 12 strains and the *ipf*A in one strain.

Significance: The results suggested that the majority of the strains, isolated from the peanut supply chain in Brazil, are not multidrug resistant and carry relevant virulence factors into the *Salmonella* genus. Therefore, the study increases the knowledge of the epidemiology and molecular basis of antibiotic-resistance and virulence of *Salmonella* recovered in low water activity food.

P3-19 Evaluation of Choridic Acid Cross-protection in *Salmonella* Typhimurium Submitted to Long-term Desiccation Stress in Peanuts

Flávia Prestes, Karina Bosqui, Ana Paula Pereira and **MARISTELA DA SILVA DO NASCIMENTO**

University of Campinas (UNICAMP), Department of Food Technology, Faculty of Food Engineering (FEA), Campinas, Brazil

Introduction: The capacity of *Salmonella* to survive in the food supply chain and in the gastrointestinal tract, is partly due to defense mechanisms that are activated during stress conditions. Preexposure of microorganisms to an unfavorable environment can select resistant cells and promote cross-protection to different types of stress.

Purpose: Evaluate the behavior of *Salmonella* Typhimurium against acid stress conditions after being recovered from roasted peanuts stored for a long time.

Methods: Two types of *Salmonella* Typhimurium ATCC 14028 inoculum were used: one cultivated in brain heart infusion broth (control) and the other recovered from roasted peanuts stored at 28°C over 550 days. The tests were performed in tryptic soy broth with differential pH adjusted with choridic acid. *Salmonella* count was determined on XLD agar every 60 min until four hours.

Results: The cells recovered from peanuts had similar behavior ($P>0.05$) to the control cells during four hours of storage at pH 7.2, with growth of *ca.* 1.0 log cfu/g. At pH 4.5, there was a statistical difference ($P<0.05$) between the samples from three hours on. Cells exposed to desiccation stress did not show growth throughout four hours, whereas an increase of 0.8 log cfu/g in the control sample was observed. At pH 3.5 the control remained stable throughout the treatment, while the cells recovered from peanuts showed reduction up to 0.8 log cfu/g. At pH 3.0, the *Salmonella* count was reduced to below the limit of detection (1.0 log cfu/g) after one hour for inoculum recovered from peanuts and after three hours for control.

Significance: *Salmonella* cells exposed to low water activity environment had high sensitivity to acid stress condition.

P3-20 Retention of Viability of *Salmonella* in Sucrose as Affected by Type of Inoculum, Water Activity, and Storage Temperature

DAVID A. MANN, Larry R. Beuchat, Christine A. Kelly and Ynes R. Ortega

University of Georgia, Griffin, GA

Introduction: Outbreaks of salmonellosis have been associated with consumption of high sugar, low water activity (a_w) foods stored for several months after manufacture. Factors affecting survival of *Salmonella* in sucrose in the absence of other food components have not been described.

Purpose: The objective of this study was to determine the effect of a_w and storage temperature on survival of *Salmonella* in dry- and wet-inoculated sucrose.

Methods: Sucrose (a_w 0.24 to 0.26 and a_w 0.44 to 0.54) was dry- and wet-inoculated with low (2.3 to 2.9 log CFU/g) and high (5.2 to 5.8 log CFU/g) populations of a five-serotype mixture of *Salmonella* and stored at 5°C and 25°C for up to 36 weeks. Duplicate samples representing all combinations of test parameters, in two independent trials, were analyzed for the presence (by enrichment) and number of *Salmonella* surviving for 0, 1, 3, 12, 18, 26, 28, and 36 weeks.

Results: Initial high counts in dry-inoculated sucrose stored at 5°C for 36 weeks decreased by 0.7 log CFU/g. Counts decreased (>3.7 log CFU/g) significantly ($P\leq0.05$) in sucrose stored at 25°C. Inactivation rates in wet-inoculated sucrose were similar to those in dry-inoculated sucrose. Survival of *Salmonella* was not markedly affected by a_w. *Salmonella* survived in low-inoculum sucrose stored at 5°C for 36 weeks, regardless of a_w or type of inoculum. In dry-inoculated sucrose (a_w 0.54) and wet-inoculated sucrose (a_w 0.24) stored at 25°C, *Salmonella* survived for up to 12 and 26 weeks, respectively.

Significance: The ability of *Salmonella* to survive in sucrose for extended periods of time highlights the need to prevent postprocessing contamination. Consideration should be given to routinely monitoring sucrose for the presence of *Salmonella*, particularly if it is intended for use as an ingredient in foods not receiving a treatment that would be lethal to the pathogen.

P3-21 Validation of Extrusion Processing as an Inactivation Step for *Salmonella* in Low-moisture Food

Tushar Verma[1], **JEYAMKONDAN SUBBIAH**[1], Andreia Bianchini[1], Jayne Stratton[1], Xinyao Wei[1], Soon Kiat Lau[1], Harshavardhan Thippareddi[2], Nathan Anderson[3] and Kent Eskridge[1]

[1]University of Nebraska-Lincoln, Lincoln, NE, [2]University of Georgia, Athens, GA, [3]U.S. Food and Drug Administration, Bedford Park, IL

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Introduction: Recent increase in the number of outbreaks/recalls due to *Salmonella* in low moisture foods has resulted in greater need for development and validation of process controls to assure its microbiological safety. Further, Food Safety Modernization Act (FSMA), Preventive Control rule requires food processors to validate their process controls ensure food safety.

Purpose: The objective of the study was to develop a response surface model to predict *Salmonella* inactivation in oat flour, as affected by product composition and process parameters.

Methods: Whole grain oat flour was adjusted to different moisture content (MC, 14 to 26% w.b.) and fat content (FC, 5 to 15%). Oat flour was inoculated with five-strain cocktail of *Salmonella* to attain ca. seven log CFU/g. Inoculated material was then extruded in a laboratory-scale single screw extruder running at different screw speeds (SS, 75 to 225 rpm) and different temperatures (T, 65 to 85°C) without a die. Samples were collected once

steady-state conditions were attained, cooled, stored under refrigeration and *Salmonella* populations were determined. Samples were plated on both nonselective media (tryptic soy agar with yeast extract, TSAYE) and selective media (TSAYE overlaid with xylose lysine deoxycholate), incubated at 37°C for 24 h. A central composite 2nd order incomplete block design was used, with the central point replicated six times.

Results: The T showed the highest effect in microbial reduction. MC showed a significant quadratic effect whereas increase in FC resulted in significant lower reduction. The SS did not play a major role, but it had an interactive effect. The response surface for inactivation of *Salmonella* spp. in selective media is: $\log(N_t/N_o)$=-7.9212+0.342*T-0.268*FC-1.007*MC+0.0762*SS-0.0011*SS*T+0.0277MC^2 (R^2=0.84).

Significance: Even under worst conditions, a significant reduction of *Salmonella* was achieved, which indicates that extrusion is an effective preventive control. Also, the developed model can be used to identify process conditions for different product matrices to achieve a desired reduction of *Salmonella*.

P3-22 Evaluation of Methods for Inoculating Powdered Milk and Soy Flour with *Salmonella enterica* Serovar Typhimurium LT2, *Enterococcus faecium,* and *Cronobacter sakazakii*

JUSTIN WIERTZEMA, Christian Borchardt and David Baumler
University of Minnesota, Saint Paul, MN

Introduction: *Salmonella* and *Cronobacter* are two microorganisms of concern in low water activity (a_w) powdered products; this is due to their ability to remain viable for long periods of time. In order to develop a method for sterilization of powdered products, first, a method for inoculating low a_w powders must be developed. There are a few existing methods, but most use a carrier molecule or have only tested one organism on powdered foods.

Purpose: The purpose of this study was to evaluate two low a_w inoculation methods and develop a methodology that does not utilize a carrier molecule and works for multiple bacterial species.

Methods: *Salmonella enterica* serovar Typhimurium LT2, *Salmonella* surrogate *Enterococcus faecium*, and *Cronobacter sakazakii* were inoculated onto both nonfat dry milk (NFDM) and soy flour using one of two inoculation methods. The first inoculation method involved adding bacterial pellets to powders, directly, and homogenizing. Inoculation method two utilized a chromatography reagent sprayer to inoculate powder.

Results: Method one achieved a significantly (P<0.05) higher inoculum level than method two in both NFDM and soy flour after four separate tests with each powder. Method one achieved inoculum log levels (CFU/g) of 9.8±0.45 in NFDM and 9.9±0.19 in soy for *C. sakazakii*; 7.4±0.35 NFDM and 8.2±0.56 soy for *S. enterica*; and 8.8±0.41 NFDM and 9.1±0.32 soy for *E. faecium*. Method two achieved inoculum levels of 9.9±0.19 in NFDM and 8.3±0.28 in soy for *C. sakazakii*; 6.3±0.22 NFDM and 6.9±0.19 soy for *S. enterica*, and 9.1±0.32 NFDM and 6.9±0.59 soy for *E. faecium*.

Significance: The methods evaluated showed that inoculation of low a_w powders with various microorganisms does not require a carrier molecule and are suitable for inoculation of various foodborne pathogen or surrogate organisms in powdered foods.

P3-23 Survival of *Salmonella* spp. and *Listeria monocytogenes* in Hot Cocoa Drink Mix When Prepared Using Common Reconstitution Methods

ASHLEY CUNNINGHAM and Kari Sweeney
Conagra Brands, Omaha, NE

Introduction: *Salmonella* spp. and *Listeria monocytogenes* are known biological hazards in milk powders and cocoa products. Proper preventive controls need to be in place. Both of these ingredients are commonly used in the formulation of hot cocoa drink mixes. Common reconstitution methods for these drink mixes use hot water or milk. However, consumer studies indicate variability in temperatures for the consumption of hot beverages. Due to such a high degree of inconsistency of beverage temperature preference, little is known about the lethality to *Salmonella* spp. and *L. monocytogenes* when cocoa is prepared using these methods.

Purpose: The purpose of this project was to examine the lethality of *Salmonella* spp. and *L. monocytogenes* in hot cocoa drink mix using common reconstitution instructions.

Methods: *Salmonella* spp. and *L. monocytogenes* inoculated nonfat dry milk was added to hot cocoa drink mix and reconstituted using 177.4 ml preheated water or milk at 120, 160, 180, 200, and 210°F in a static state or under mechanical shaking for 30, 60, and 90 seconds. Recovery data was log-transformed and analyzed using ANOVA at P<0.05.

Results: *Salmonella* and *L. monocytogenes* were significantly decreased with the shake method compared to the hold method and both pathogens decreased with an increase in time and temperature. Inactivation kinetics in both pathogens remained the same across all replications. While a significant decrease of both *Salmonella spp.* and *L. monocytogenes* began at 180°F at 30 s, a temperature of at least 180°F with shaking for at least 90 s was required to achieve a minimum four-log CFU/g reduction.

Significance: A microbiological contamination risk may exist in milk or cocoa powders used in drink mixes. As shown by this study, the risk is not easily eliminated by simply following common reconstitution instructions.

P3-24 Influence of Water Activity on *Listeria monocytogenes* Growth in Brain Heart Infusion Agar

GUIOMAR DENISSE POSADA-IZQUIERDO[1], Arícia Possas[2], J.C.C.P. Costa[1], Fernando Pérez-Rodríguez[1], Antonio Valero[1] and Rosa Maria Garcia-Gimeno[1]
[1]University of Cordoba, Cordoba, Spain, [2]University of Cordoba, Department of Food Science and Technology, Cordoba, Spain

Introduction: *Listeria monocytogenes* has a significant impact on food safety for being a highly adaptable microorganism that can persist in meat products with a wide range of water activities (a_w) and salt concentrations such as the Spanish chorizo sausage.

Purpose: The aim of this study was to evaluate the growth capacity of *L. monocytogenes* in brain heart infusion agar (BHIA), considered as a simulated cured meat system, with different a_w values.

Methods: A factorial design consisting on the addition of different concentrations of glycerol (0, 5.0, 10.0, 15.0, 20.0 % v/v) and NaCl salt (0, 2.5, 5.0, 7.5, 10.0 % w/v) to BHIA was conducted in order to adjust the a_w values. The culture mediums prepared according to the factorial design were pour plated in petri dishes previously inoculated with one ml of a four-strain *L. monocytogenes* cocktail at a concentration of nearly 10 CFU/ml. The plates were incubated at 37°C and the incubation time varied according to the lag phase observed in each case. The number of cells were determined by count in plate methodology.

Results: The a_w values of BHIA ranged from 0.99 to 0.83 and decreased with the increase in salt and glycerol concentrations. In general, *L. monocytogenes* was able to grow in BHIA with a_w values higher than 0.92. The lag time increased with the increase in salt and glycerol concentrations, varying between 24 and 96 h. Salt concentrations higher than 7.5% did not allow *L. monocytogenes* growth probably due to cells osmotic stress. In BHIA plates with 20% of glycerol, growth was verified only when salt was not added.

Significance: The data presented in this study may help to stablish and evaluate the growth/no growth boundaries of *L. monocytogenes* in agar simulating meat systems, as a function of a_w and salt concentrations.

P3-25 Survival of Shiga-toxigenic *Escherichia coli* in Flour

Paula Bauer[1], **VALERIE NETTLES**[2], Stuart Gorman[2] and Faith Critzer[2]
[1]*University of Hohenheim, Stuttgart, Germany,* [2]*University of Tennessee, Department of Food Science, Knoxville, TN*

Introduction: Historically, dry ingredients have been viewed as lower risk due limited ability for survival given the low water activity (a_w). This paradigm is changing, however, given outbreaks of *Salmonella* and Shiga toxigenic *Escherichia coli* (STEC) with a_w controlled ingredients and foods. Although a low a_w can inhibit the growth of bacteria, certain foodborne pathogens such as STEC can persist in these products for extended periods of time. Survival data for STEC in dry ingredients, such as flour, is necessary for proper risk assessment.

Purpose: Evaluate the ability of various STEC serotypes to survive in dry flour.

Methods: Representative serotypes from four STEC serogroups (O45, O103, O145, and O157) were used to inoculate separate samples of sterilized all-purpose flour. Flour was stored in an environmental chamber (25°C, RH=57%) to simulate a dry storage environment, then sampled routinely for water activity measurement and enumeration of STEC. All experiments were independently replicated (n=9).

Results: After five weeks of storage, O45 and O145 serotypes showed the greatest survival in flour with log reductions of 0.63 (±0.47) log CFU/g and 0.77 (±0.48) log CFU/g, respectively. Populations of O157 and O103 had significantly reduced (P<0.05) over five weeks with 1.2 (±0.29) log CFU/g and 1.2 (±0.26) log CFU/g, respectively. After six weeks, all serotypes except O45 were below the limit of detection (50 CFU/g), but positive after enrichment. After seven weeks, all serotypes were only positive by enrichment.

Significance: These data suggest that STEC can remain viable over many weeks stored at room temperature in a low a_w environment. More studies will be needed to further characterize the survival of other STEC serotypes in this environment for longer periods of time.

P3-26 Effect of Added Water and Steam on the Heat Resistance of *Salmonella* Enteritidis Phage Type 30 Surrogate*, Enterococcus faecium*, on Blanched Peanuts, Cashews, Pumpkin Seeds, Red Skin Peanuts, and Walnuts

BUKOLA ONARINDE, Pauline Lovatt, Yunus Khatri and Gerrit Meerdink
University of Lincoln, Lincoln, United Kingdom

Introduction: It is well known that microorganism in dry environment have significantly higher heat resistance than similar microorganisms in an environment with higher available water.

Purpose: The aim of this experiment is to determine the effect of added water and steam on the heat resistance of *Enterococcus faecium* inoculated on selected low moisture products (blanched peanuts, cashews, pumpkin seeds, red-skin peanuts, and walnuts).

Methods: Products were artificially inoculated with *E. faecium* and dried for 24 hours. With the use of a pilot scale batch oven, the heat resistance of *E. faecium* inoculated on the products was evaluated under three different conditions, (1) dry products exposed to dry heat at 160°C for 25 minutes, (2) wet products (dry product with 10% sterile water added) exposed to dry heat at 160°C for 25 minutes and (3) dry products exposed to steam at 160°C for 10 minutes.

Results: After artificial inoculation and drying level of *E. faecium* on products (n=6) prior to heat treatment were 8.56 log CFU/g (blanched peanuts), 9.00 log CFU/g (cashew nuts), 8.70 log CFU/g (pumpkin seeds), 8.30 log CFU/g (red-skin peanuts) and 7.26 log CFU/g (walnuts). The result of the heat treatment showed a significance different (P≤0.01) between the use of dry heat and the use of wet product and steam. After heat treatment log reduction (n=6) achieved for (1) dry products exposed to dry heat ranged from 4.98 to 6.11 log CFU/g, (2) wet products exposed to dry heat ranged from 6.95 to 7.70 log CFU/g and (3) dry products exposed to steam ranged from 6.95 to 7.60 log CFU/g.

Significance: The result obtained in this study confirms that dry microbial cells have significantly greater heat resistance that cells in an environment where water activity is close to one.

P3-27 Antimicrobial Effect of Nanocomposite Films Made of Cloisite 30B-Metal Nanoparticle in Soy Burger

FARANAK BEIGMOHAMMADI[1], Seyed Hadi Peighambardoust[2] and Seyed Jamaledin Peighambardoust[3]
[1]*Food Science and Technology Department, Agriculture College, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran, Kermanshah, Iran,* [2]*University of Tabriz, College of Agriculture, Department of Food Science, Tabriz, Iran,* [3]*University of Tabriz, Faculty of Chemical and Petroleum Engineering, Tabriz, Iran*

Introduction: Nowadays, active packaging is a novel way to protection of food.

Purpose: The objective of this research was to introduce a new packaging for food.

Methods: This study was undertaken to investigate the ability of different kinds of nanocomposite films made of cloisite-30B with different percentages of silver and copper oxide nanoparticles incorporated into a low-density polyethylene (LDPE) polymeric matrix by a melt mixing method, in order to inhibit the growth of microorganisms in soy burger.

Results: The number of surviving cell of the total count was decreased by 3.61 log and mold and yeast diminished by 2.01 log after eight weeks storage at 18±0.5°C below zero. Pure LDPE did not have an antimicrobial effect. A composition of 1.3% cloisite 30B-Ag and 2.7% cloisite 30B-CuO for total count and 0% cloisite 30B-Ag and 4% cloisite 30B-CuO for yeast and mold gave optimum points in the combined design test in Design Expert 7.1.5. Suitable microbial models were suggested for retarding the above microorganisms growth in soy burger. To validate the optimum point, the difference between the optimum point of nanocomposite film and its repeat was shown to be not significant (P<0.05) by one-way ANOVA analysis using SPSS 17.0 software; the difference was significant for pure film. Migration of metallic nanoparticles into a food stimulant was within the accepted safe level.

Significance: The results for this packaging, as analyzed by microbial models, showed that the film could be applied to soy burger and similar products to decrease contamination.

P3-28 The Growth/Survival of *Salmonella* on Waxberry under Different Storage Temperatures and Package Materials

XINGNING XIAO[1], Wen Wang[2], Weihuan Fang[3], Yingchun Fu[1] and Yanbin Li[4]

[1]Zhejiang University, College of Biosystems Engineering and Food Science, Hangzhou, China, [2]Institute of Quality and Standard of Agricultural Products, Zhejiang Academy of Agricultural Sciences, Hangzhou, China, [3]Zhejiang University, College of Animal Sciences, Hangzhou, China, [4]University of Arkansas, Department of Biological and Agricultural Engineering, Fayetteville, AR

Introduction: Waxberry *(Myrica rubra)* is one of the main economic fruits in Zhejiang Province, China. The contamination of *Salmonella* on fresh waxberry poses a threat to the public. Storage temperature abuse and inappropriate packaging materials could cause bacterial growth, which is considered critical for ensuring food safety. Currently, the growth/survival of *Salmonella* on fresh waxberry at different temperatures and with different package materials during storage has not been fully studied.

Purpose: The objective of this study was to determine the effects of temperature and packaging materials on the growth/survival of *Salmonella* on waxberry during storage.

Methods: Waxberry samples inoculated with *Salmonella* spp. cocktails (ATCC 14028, 50335) were storied at 4°C, 16°C, and 33°C for 72 h, using two package materials of plastic sealing bags and preservative film, respectively. Bacterial populations were enumerated every eight hours. Primary models (log-linear, Gompertz, and Weibull) were used to fit curves, and statistic criteria of goodness-of-fit such as mean square error, RMSE were used for model evaluation.

Results: A reduction of two log CFU/g of *Salmonella* spp. on waxberry was observed after 72 h storage at 4°C, while a more rapid decrease was found with the preservative film packaging with plastic sealing bags ($P<0.01$). The lag phase of *Salmonella* spp. on waxberry was up to 40 h at storage temperatures of 16°C and 33°C and then an increase of one to two log CFU *Salmonella* spp./g was observed. The prevention of bacterial growth with the packages with preservative film was greater than that with plastic sealing basg ($P<0.01$). The log-linear model performed better for describing the bacterial growth/survival curves at three different temperatures, as demonstrated by the statistical indices ($R^2>0.9$, $P<0.05$, RMSE<0.15).

Significance: This study provided a predictive model for evaluation of the storage methods for waxberry.

P3-29 A Preliminary Report: Impact of Reusing Jute Bags on Aflatoxin Contamination of Maize

CUI WANG[1], Feng Xu[1], Xiaofeng Hu[1], Ali Pinjari[2], Jing Ren[1] and Robert Baker[1]

[1]Mars Global Food Safety Center, Beijing, China, [2]Mars International India Pvt. Ltd, Hyderabad, India

Introduction: Jute bags are used, worldwide, as packages for many raw materials. In India, the jute bags are frequently reused without specific treatments. There was a considerably high rejection rate (30% to 50%) of maize loads in India due to aflatoxin contamination. Aflatoxins, fungi secondary metabolites, have been confirmed as carcinogenic and a major food safety risk in both foodstuffs and feedstuffs.

Purpose: The focus of the study was to understand and evaluate the impact of reusing jute bags on aflatoxin contamination.

Methods: New and reused Jute bag samples were collected from India. *Aspergillus flavus* and *Aspergillus parasiticus* groups of fungi were isolated from these samples and further screened for toxigenic fungi by molecular and chemical methods. The aflatoxin concentration from new and reused jute bags and the maize inside were determined by ELISA and LC methods. We assessed the relationship between population of the fungi, aflatoxin concentration of jute bag samples, and aflatoxin risk of maize in India.

Results: The results showed an aflatoxin risk profile of reusing jute bags. The reused jute bags were divided into released and rejected groups, based on whether the aflatoxin concentration of maize inside exceeded 12 ppb or not. Both the total and aflatoxigenic *Aspergillus* fungi population from rejected jute bags were higher than from released jute bags. New jute bags contained very little toxigenic fungi. Furthermore, the aflatoxin concentration determined from reused jute bags indicated a similar trend and cross-contamination risk. The rejected jute bags reached high aflatoxin concentrations of >8.83 ppb and >93 times higher toxigenic fungi population levels than released ones.

Significance: This systematic study uncovered the potential risk of aflatoxigenic fungi and cross-contamination caused by reusing jute bags. This information will facilitate aflatoxin prevention and management in India and other countries.

P3-30 Antimicrobial Food Packaging with Olive Leaf Extract

TONY JIN, Yanhong Liu and Lindsay McKeever

U.S. Department of Agriculture-ARS, Eastern Regional Research Center, Wyndmoor, PA

Introduction: Antimicrobial food packaging has demonstrated the capability to enhance food safety and extend food shelf-life. Antimicrobial packaging with natural plant extracts is more desirable to food manufacturers and consumers, since plant extracts are generally recognized as safe and may benefit human health.

Purpose: The purpose of this study was to develop antimicrobial packaging films using olive leaf extract as an antimicrobial agent and investigate their effectiveness against foodborne pathogens.

Methods: Olive leaf extract (OLE) was made from fresh olive leaves using an alcohol extraction method followed by vacuum-freeze-drying. Commercial OLE powders were also used to compare the effectiveness compared of the laboratory-prepared OLE. Antimicrobial (AM) film was developed by mixing 6% OLE, 6% chitosan, and 2% organic acid and then drying the mixture for 24 h. The antimicrobial efficiency of AM film samples was evaluated in peptone water and apple juice (ratio of 0.5 g OLE per each 10 ml liquid sample), which were inoculated with *Escherichia coli* K-12 (surrogate of *E. coli O157:H7*) and *Listeria innocua* (surrogate of *Listeria monocytogenes*). The surviving microbial populations were determined at 24 h and 48 h after film treatments.

Results: In peptone water, the AM film reduced *E. coli* and *L. innocua* populations from 8.2 and 7.8 CFU/g, respectively, to <1 log/g after 24 h. No bacterial cells were detected after 48 h. In apple juice, the AM film reduced *E. coli* populations from 7 to <1 log/g after 24 h and reduced *L. innocua* populations from 7 to 1.5 CFU/g at 24 h and <1 CFU/g after 48 h. There were no significant ($P>0.05$) differences in antimicrobial efficiency between the commercial and laboratory-prepared OLE samples.

Significance: The results showed that AM films with OLE were highly effective against both gram-positive and gram-negative bacteria in liquid products and that the films have the potential to reduce pathogenic contaminants in solid food products.

Poster

P3-31 Inactivation of *Escherichia coli* O157:H7 and *Salmonella* spp. on Fresh Strawberries by Antimicrobial Washing and Coatings

TONY JIN[1], Mingming Guo[2] and Joshua Gurtler[1]
[1]*U.S. Department of Agriculture-ARS, Eastern Regional Research Center, Wyndmoor, PA,* [2]*University of Delaware, Newark, DE*

Introduction: Strawberries have been associated with an outbreak of hemorrhagic colitis due to pathogenic contaminations. Our previous studies showed that a single treatment (antimicrobial washing or coating) was effective in inhibiting foodborne pathogens. However, their combination might provide additive or synergistic effects for strawberries that have not been further investigated.

Purpose: This study compared the effects of antimicrobial washing, surface coating, and their combination on reduction and inhibition of artificially inoculated pathogens and natural microflora on strawberries and their impacts on the quality of treated strawberries stored at 4°C.

Methods: Strawberries were inoculated with *Escherichia coli* O157: H7 and *Salmonella* spp., washed in an antimicrobial solution (90 ppm peracetic acid for 1 min), coated with a solution (1% chitason+1% allyl isothiocyanate (AIT)+1% acids), or treated in their combination (washing+coating). Treated samples were placed in PET boxes (8 oz) and stored at 4°C for three weeks. The survival and growth of inoculated pathogens and natural microflora were determined. The appearance, weight loss, color, and firmness of the strawberry were also evaluated during storage.

Results: Antimicrobial washing or coating achieved an average reduction of 0.5 to 3.0 log CFU/strawberry of *E. coli* O 157:H7 and *Salmonella* spp., natural bacteria, and yeast and mold; and also significantly (P<0.05) inhibited their growth over a three week period. The combined treatment had an additive antimicrobial effectiveness. After three weeks, the combined treatment reduced *E. coli* O157:H7 and *Salmonella* spp. from 6.6 to 2.5, natural bacteria from 3.8 to 1.2, and yeast and mold from 4.9 to 1.1 log CFU/strawberry. In addition, the combined treatment prevented weight loss and maintained the firmness, color, and fresh appreance of strawberries during storage as compared to other strawberry samples.

Significance: The results indicated that this is a viable antimicrobial method to improve the microbiology safety and quality of strawberries from field to table.

P3-32 A Novel Bioactive Film Based on Pink Pepper Extract Combined with Modified Atmosphere Packaging Inhibits Spoilage Microorganisms of Atlantic Salmon Fillets

Thais Cardoso Merlo[1], Mariana Vieira Santana[2], Caio Cesar de Sousa Ribeiro[1], Giovana Verginia Barancelli[1], Patricia Santos Lopes[2], Cristiana Maria Pedroso Yoshida[2], **ANNA CECILIA VENTURINI**[2] and Carmen Josefina Contreras-Castillo[1]
[1]*Universidade de São Paulo, Departamento de Agroindustria, Alimentos e Nutrição, ESALQ, Piracicaba, Brazil,* [2]*Departamento de Ciências Farmacêuticas, Universidade Federal de São Paulo, Diadema, Brazil*

Introduction: Pink pepper (*Schinus terebinthifolius Raddi*) is a rich source of phenolic compounds with antioxidant and antimicrobial effects and can increase shelf-life and food safety of fresh salmon, which is greatly limited by microbial deterioration.

Purpose: The aim of this work was to develop an active biobased chitosan film with incorporated pink pepper extract to extend the microbiological quality of salmon fillets stored under modified atmosphere (MA; 100% CO_2) at 2°C.

Methods: Fillets, with and without skin, were packed in MA and stored at 2±1°C according to the following treatments: without chitosan film (TC), with chitosan film (TFQ), and with chitosan film incorporated with pink pepper extract (TFQPR). Every week, nine trays of each treatment were analyzed for aerobic mesophilic and psychrotrophic bacteria, lactic acid bacteria (LAB), thermotolerant bacteria and total coliform. Differences between means were compared using ANOVA.

Results: The aerobic microflora increased with storage time for all treatments. After 28 days of storage, aerobic counts of TFQ, TFQPR, and TC (fillets with skin) samples did not exceed six log CFU/g, considered the upper acceptability limit for fresh fish. The control treatment (TC), composed of fillets without skin, had higher mesophilic counts (near to seven log CFU/g) in comparison to the others treatments (P<0.05), which demonstrated the additional antimicrobial protection of active biobased chitosan films against deterioration of salmon fillet. The anoxic atmosphere favored the selection of LAB, but the counts (<six log CFU/g) were not enough to compromise the microbiological quality of TFQ and TFQPR fillets. Thermotolerant bacteria and total coliform microorganisms remained within the limits established by ICMSF (<10^3 MPN/g) in all treatments.

Significance: Results observed in this study provide a new insight for active biobased chitosan film/pink pepper as a potential antimicrobial agent for fresh fish. Such extended storage time would not be obtained with poor initial product quality.

P3-33 Ultraviolet-activated Titanium Dioxide Nanocomposite Polymer Films: Characterization and Antimicrobial Efficacy against *Escherichia coli* O157:H7

JING XIE and Yen-Con Hung
University of Georgia, Griffin, GA

❖ ***Developing Scientist Competitor***

Introduction: Prepackaged minimally processed food poses a microbiological safety concern due to the high risk of cross-contamination during processing. Titanium dioxide (TiO_2) nanoparticles (NPs) are photocatalysts that have shown great effectiveness in inactivation of pathogenic microorganisms owing to strong oxidizing capabilities during photocatalytic reactions. Incorporating TiO_2 NPs into packaging material may effectively inhibit bacterial growth in prepackaged food products.

Purpose: The objectives of this work were to develop plastic films incorporating TiO_2 NPs using different polymer materials, to perform characterization of these films, and to determine their antimicrobial activity against *Escherichia coli* O157:H7.

Methods: Biodegradable polymers (cellulose acetate (CA), polycaprolactone (PCL) or polylactic acid (PLA)) and TiO_2 nanoparticles (1, 3, and 5%) were mixed in organic solvent and casted in glass petri dishes. Optical and physical properties of the films were assessed by measuring the thickness, color, ultraviolet-visible (UV-Vis) absorption spectra and UV-A transmission. Photocatalytic activity of the films was examined by degradation of methylene blue. To test the antimicrobial activity of the films against *E. coli* O157: H7, bacterial inoculum was added to the film surface and a covered with a transparent plastic film (4 by 4 cm^2). Inoculated films were placed under UV-A light (intensity at 1.30±0.15 mW/cm^2) for two hours.

Results: TiO_2 NPs incorporated polymer films presented uniform TiO_2 distribution and even color. PCL and CA films with 5% TiO_2 were able to degrade methylene blue in solution by 72% and 53%, respectively; however, PLA films did not show significant photocatalytic activity. CA film incorporated with 5% TiO_2 NPs had the highest bactericidal activity and achieved 1.82 log CFU/ml reduction. Whereas, PCL and PLA composite films did not show significant bactericidal property (less than 0.6 log reduction).

Significance: This study indicated that UV-activated TiO_2 CA films could potentially be used to inactivate microorganisms in packaging during storage and distribution.

Poster

P3-34 Cold Plasma Inactivation of *Salmonella* in Prepackaged, Mixed Salads is Influenced by Cross-contamination Sequence

SARAH HERTRICH[1], Glenn Boyd[2], Joseph Sites[3] and Brendan Niemira[1]

[1]U.S. Department of Agriculture-ARS, Wyndmoor, PA, [2]U.S. Department of Agriculture-ARS-ERRC-FSIT, Wyndmoor, PA, [3]U.S. Department of Agriculture-ARS-FSIT, Wyndmoor, PA

Introduction: The increased demand by consumers for convenient food products has led to an increased production of prepackaged and ready-to-eat food products. Most of these products including fresh produce rely mainly on surface disinfection and other traditional approaches to ensure shelf-life and safety. Novel processing techniques, such as cold plasma, are currently being investigated to enhance the safety and shelf-life of prepacked foods.

Purpose: The purpose of this study was to determine the effects of cold plasma corona discharge on the inactivation of *Salmonella* on prepackaged, tomato and lettuce mixed salads. Two different inoculation methods were evaluated, as well as the cross-contamination of *Salmonella* from cherry tomatoes to lettuce and vice versa following cold plasma treatment.

Methods: In separate studies, either cherry tomatoes (10 g) or romaine lettuce (10 g) were inoculated with a *Salmonella* cocktail (*Salmonella* Anatum, *S*almonella Enteriditis PT30, and *Salmonella* Stanley) and placed into a commercial PET plastic container and thoroughly mixed together with its uninoculated counterpart. Mixed salads were allowed to dry in a biosafety cabinet for one hour. Samples were treated with 35 kV cold plasma corona discharge inside plastic containers for three minutes. Samples were stomached and serially diluted in BPW. Dilutions were plated onto APC Petrifilm and incubated for 18 h at 37°C.

Results: After three minutes of cold plasma treatment, *Salmonella* was significantly reduced (P=0.002;α=0.05) in the mixed salad (0.53±0.31 log CFU/g) when cherry tomatoes were the inoculated material, but not when lettuce was the inoculated material. In both scenarios, recoverable *Salmonella* populations increased on lettuce and decreased on tomatoes following cold plasma treatment.

Significance: Cold plasma can kill *Salmonella* in a in a prepackaged, mixed salad, with efficacy dependent on the nature of contamination and on the surface topography of the contaminated commodity.

P3-35 Cucumber Waxing Significantly Enhances the Survival of *Salmonella enterica* Serovar Newport on the Fruit Surface

MARY THERESA CALLAHAN and Shirley Micallef

University of Maryland, College Park, MD

Introduction: A 2014 multistate foodborne illness outbreak caused by *Salmonella enterica* serovar Newport was responsible for 275 illnesses and traced back to contaminated cucumbers. In 2015, an outbreak caused by *Salmonella* Poona-contaminated cucumbers caused over 900 illnesses in the United States. Few studies have investigated the persistence of *Salmonella* on the surface of cucumbers. Further, waxing is a common practice to preserve the water content and hence the turgidity of fruit. However, little is known about the effect of wax on epiphytic bacteria, including enteric pathogens.

Purpose: This study investigated the survival of *Salmonella* Newport on the surface of six cucumber cultivars of varying surface topographies. The effect of a commercial wax on the persistence of *Salmonella* Newport was also studied.

Methods: *Salmonella* Newport adapted for rifampicin resistance (~6.5 log CFU/ml) were spot inoculated (100 µl) on the surface (~2 cm spot) of cultivars Marketmore, Patio Snacker, Corinto (rough, spined varieties), Bella, Pepinex, and Summer Dance (smooth, spineless varieties). Inoculated spots of half the samples of each cultivar were covered with 20 µl wax using a sterile toothbrush. Cucumbers were held at room temperature for 24 h. *Salmonella* Newport was enumerated by direct plate count onto tryptic soy agar amended with 50 µg/ml rifampicin.

Results: Waxing significantly lessened population decline on cucumber surfaces after 24 h (P<0.01). While there was ~ three log CFU/g decline in populations on unwaxed cucumbers, the decline on waxed cucumbers was ~ two log CFU/g. For both treatments, cultivars with rough surfaces supported smaller populations than smooth cultivars (P<0.01).

Significance: This study provided evidence that commercial wax may enhance the risk of foodborne illness by enhancing persistence of *Salmonella*. It also provides evidence for cucumber cultivar differences in *Salmonella* survival; where cucumbers with spines or ridges may harbor smaller populations than smooth-skinned cultivars.

P3-36 The Effect of Pear Firmness on the Transfer of *Salmonella* during Mechanical Slicing

HAMOUD ALNUGHAYMISHI and Elliot Ryser

Michigan State University, East Lansing, MI

❖ ***Developing Scientist Competitor***

Introduction: Numerous studies have examined the extent of microbial cross-contamination during preparation of fresh-cut produce, however few investigators have assessed the impact of specific processing parameters on pathogen transfer.

Purpose: This study aimed to evaluate the impact of pear firmness on transfer of *Salmonella* during mechanical slicing.

Methods: Locally purchased pears were categorized as firm (54 to 60 N), medium (31 to 42 N) or soft (<31 N) based on force measurements from a texture analyzer equipped with an eight mm diameter probe. The desired firmness was achieved by incubating the pears at 20°C for no more than 72 h. Pears were individually dip-inoculated with a three-strain *Salmonella* cocktail (Montevideo, Poona, Newport) at ~ five log CFU/cm^2 and then air-dried for one hour. Thereafter, one pear was sliced to contaminate the NEMCO vertical slicer followed by 15 uninoculated pears. Three slices per pear were homogenized by stomaching, appropriately diluted and surface plated on trypticase soy agar with yeast extract containing 0.05% ferric ammonium citrate and 0.03% sodium thiosulfate to enumerate healthy and injured *Salmonella.*

Results: Based on triplicate experiments, samples from the 1st , 9th, and 15th firm pears yielded average *Salmonella* populations of 2.4±0.1, 0.8±0.5, and 0.4±0.1 log CFU/cm^2, respectively, which were significantly lower (P<0.05) than medium (3.1±0.2 , 1.2±0.4, and 0.6±0.1 log CFU/cm^2) and soft pears (3.6±0.1 , 1.5±0.2, and 1.1±0.3 log CFU/cm^2). In addition, the total number of *Salmonella* cells transferred was statistically higher for firm (P<0.05) as compared to medium and soft pears.

Significance: The extent of cross-contamination of fresh produce during slicing is affected by firmness. These findings should prove useful in developing improved predictive models for bacterial transfer and expanding current risk assessments across a wider range of products.

Poster

P3-37 Reduction of *Salmonella* on the Surface of Green Skin Avocados by Antimicrobial Chemicals in a Pilot Brush Wash System

LIDIA N. VALDÉS and Michelle D. Danyluk
University of Florida, Lake Alfred, FL

❖ ***Developing Scientist Competitor***

Introduction: Little information has been reported about the presence, survival, and prevention of *Salmonella* on the whole green skin avocados.

Purpose: The objective of this work was to evaluate the efficacy of sodium hypochlorite (NaOCl), and peroxy-acetic acid (PAA) at reducing *Salmonella* on the surface of green skin avocados using a laboratory scale spray brush roller system.

Methods: Avocados were inoculated on the smooth middle section with a five-strain cocktail (seven-log CFU/avocado) of rifampicin resistant *Salmonella*, dried for one hour, and submitted to spray washing for 0, 5, 15, 30, and 60 s, using 100 ppm NaOCl (pH: 7.0) or 85 ppm PAA, and a water control. *Salmonella* populations were enumerated following removal from avocados using a rub-shake-rub in Dey/Engley (DE) neutralizing broth and spreading dilutions on tryptic soy agar (TSA) and bismuth sulfite agar (BSA) supplemented with rifampicin. Experiments of triplicate samples were repeated five times (n=15). Average log CFU/avocado reduction of *Salmonella* were calculated for each spray time and sanitizer, and compared using Analysis of Variance (ANOVA) and Tukey's HSD ($P \leq 0.05$) for each medium.

Results: *Salmonella* populations were reduced significantly more ($P \leq 0.05$) by the presence of NaOCl and PAA compared to the water control, for all treatment times on both media types; there were not significantly differences ($P > 0.05$) in *Salmonella* population reductions between NaOCl and PAA treatments in 15, 30, and 60 s. *Salmonella* populations were reduced by >3.93±0.30 log CFU/avocado with NaOCl and PAA treated for 15 s versus 2.13±0.63 log CFU/avocado reduction achieved with water.

Significance: Addition of NaOCl or PAA can significantly reduce *Salmonella* populations on the surface of green skin avocados compared to water alone, and may be used as a reduction strategy to prevent or minimize avocado contamination during postharvest practices in Florida packinghouses.

P3-38 Plant-Microbe Factors Influencing *Salmonella* Survival and Growth on Alfalfa Sprouts and Microgreens

ELIZABETH REED, Christina Ferreira, Rebecca Bell, Eric Brown and Jie Zheng
U.S. Food and Drug Administration, College Park, MD

Introduction: Microgreens have not been associated with an outbreak, but are similar to sprouts, which have been associated with numerous foodborne outbreaks. Understanding the risks of *Salmonella* contamination and its effects on rhizosphere microbial communities will enable a better understanding of host-pathogen dynamics in these commodities.

Purpose: These studies were performed to examine the factors that affect *Salmonella* survival and growth on alfalfa sprouts and microgreens and to investigate the factors influencing the rhizosphere microbial communities during sprouting.

Methods: Different *Salmonella* serovars (Hartford and Cubana) at inoculation levels (10^{1}, 10^{-1}, and 10^{-2} CFU/ml) were evaluated for survival and growth on alfalfa sprouts using most probable number (MPN) method. In addition, edible/nonedible portions of microgreens and various soil types were also tested. Community level physiological profiles (CLPP) of sprout/microgreen rhizospheres were characterized with Biolog EcoPlates at different time points during sprouting

Results: Overall, inoculation level, serovar, and inoculation level by serovar interaction significantly affected the survival/growth of *Salmonella* in alfalfa sprouts ($P<0.0001$), but had no significant effect in microgreens ($P=0.57719$, and $P=0.15348$). At lower inoculation levels, Hartford was able to survive/grow better, while Cubana did significantly better than Hartford at 10^{1}CFU/ml. Soil type played an important role in *Salmonella* survival/growth in microgreens ($P=0.00946$). CLPP analysis showed significant changes in the microbial functional diversity during sprouting for alfalfa sprouts, but not for microgreens. *Salmonella* contamination did not confer significant changes in the corresponding rhizosphere microbial community in both sprouts and microgreens.

Significance: The growth and persistence of *S. enterica* on alfalfa sprouts, but not microgreens, is serovar and inoculation level dependent. CLPP analysis revealed the change in rhizosphere bacterial functional diversity was host-dependent, but independent of *Salmonella* contamination. This data will aid further design of preventive strategies of *Salmonella* contamination in sprouts and microgreens.

P3-39 Fate of *Salmonella* spp. and *Listeria monocytogenes* on the Surface of Whole Mangoes Stored at Three Temperatures

LORETTA FRIEDRICH and Michelle D. Danyluk
University of Florida, Lake Alfred, FL

Introduction: Imported mangoes have been implicated in multistate *Salmonella* spp. outbreaks in the United States of America. Scientific data establishing the behavior of pathogens on the surface of mangoes is important for industry to comply with FSMA Produce Safety Rule corrective measure requirements if agricultural water used during production does not meet the required standards.

Purpose: The purpose of this research is to evaluate the persistence of foodborne pathogens on the intact surface of whole Ataulfo, Kent, and Tommy Atkins mangoes stored at three different temperatures.

Methods: One hundred microliters of a five-strain rifampicin resistant *Salmonella* spp. or *Listeria monocytogenes* cocktail (six log CFU/mango) was spot inoculated onto the mid-section of whole fruit and dried for one hour. Fruit were stored at 12, 20, or 30 ± 2°C and sampled for up to 28 days. At each sampling point a mango was placed in a sterile bag with 10 ml of 0.1% peptone and bacterial populations were removed by a rub-shake-rub method. Pathogen populations were enumerated by plating onto selective and nonselective media supplemented with rifampicin. Experiments were replicated in duplicate three times for each variety (n=6).

Results: Populations of *Salmonella* increased over storage duration on the surfaces of Kent (0.3 to 1.1 log CFU/mango) and Tommy Atkins (0.2 to 1.4 log CFU/mango) mangoes at all temperatures. *Salmonella* populations on Ataulfo mangoes decreased at 12°C (1.6 log CFU/mango) and 30°C (0.4 log CFU/mango) but increased at 20°C (0.1 log CFU/mango). *Listeria* populations on the surface of Tommy Atkin mangoes increased at all temperatures ranging from 0.1 to 1.4 log CFU/mango.

Significance: These results imply that postharvest storage of mangoes will not result in sufficient microbial reductions to be used as corrective measures if agricultural water used during production does not meet the required standards of Produce Safety Rule.

Poster

P3-40 Characterization of a Pathogen Strain Collection to Allow for Improved Validation of Sanitizer Efficacy in the Produce Industry

ANNA SOPHIA HARRAND, Veronica Guariglia-Oropeza, Jasna Kovac, Laura Carroll and Martin Wiedmann
Cornell University, Ithaca, NY

❖ ***Developing Scientist Competitor***

Introduction: Effective control of foodborne pathogens on produce and in produce associated environments requires science-based validation of interventions and control strategies. It has previously been shown that strains and/or genetic lineages of a pathogen may differ in their ability to survive different stress conditions. We hypothesize however that pregrowth conditions have a significantly greater effect on subsequent stress survival and stress response than genetic diversity within produce relevant pathogens, including *Salmonella*, *Listeria monocytogenes*, and shiga toxin-producing *Escherichia coli* (STEC).

Purpose: To provide a produce associated strain collection for challenge and validation studies. To provide data if multistrain or multigrowth condition best capture the range of phenotypic responses to a given challenge.

Methods: A proposed strain collection was evaluated by experts for inclusion in the study. All isolates in the final collection were characterized by whole genome sequencing. A subset of five selected strains were grown under eight different stress conditions (e.g., low temperature, high salt, oxidative stress) and exposed for 45 s to 40 ppm of peroxyacetic acid, whereas strains' ability to survive intervention treatments was determined by reduction of pathogen number.

Results: The finalized collection is comprised of 23 *Salmonella* strains, 11 *L. monocytogenes* strains, 13 STEC strains, and 8 surrogate, indicator and index organisms. With three exceptions, whole genome sequencing data confirmed serotype and lineage classification of the selected isolates. *Salmonella* strains selected for sanitizer treatment show higher variance of log reduction based on pregrowth conditions (e.g., log reduction of 3.6 to 7) as compared to strain diversity (e.g., log reduction of 3.4 to 5).

Significance: These data, along with the strain collection, will facilitate selection of appropriate strains and growth conditions for challenge studies. The study will provide a resource to scientifically justify using specific strain sets or cocktails or using strains grown under specific conditions prior to challenge or validation experiments.

P3-41 Optimization of Time and Temperature of Hot Water Treatment as a Kill Step to Inactivate *Salmonella* spp. and *Escherichia coli* O157:H7 in Pecan Processing

KARUNA KHAREL[1], Achyut Adhikari[1], Charles Graham[2] and Namrata Karki[1]
[1]Louisiana State University AgCenter, Baton Rouge, LA, [2]Louisiana State University, Baton Rouge, LA

❖ ***Developing Scientist Competitor***

Introduction: Pecans harvesting includes collection of pecans after they have fallen to the ground. This increases the potential risks of foodborne pathogen contamination. Low-moisture foods like nuts require a five log reduction process of foodborne pathogens to be regarded as a kill step.

Purpose: This study evaluated the effect of hot water at various time and temperature points to establish a kill step for *Salmonella* and *Escherichia coli* O157:H7 during shelling of pecan.

Methods: Pecan samples from Louisiana farms were inoculated with a cocktail of nalidixic acid resistant strains of four serotypes of *Salmonella enterica* or five strains of *E. coli* O157:H7. *Salmonella* or *E. coli* O157:H7 inoculated pecan samples were then treated with hot water maintained at 70°, 80°, and 90°C for one, two, three, four, and five minutes. Enumeration was done in XLD agar plates for *Salmonella* and CT-SMAC plates containing nalidixic acid (50µg/ml) for *E. coli* O157:H7 .

Results: A six log reduction of *E. coli* O157:H7 was achieved within four minute of treatments at 70°C (6.5±1.0 log cfu/g), within three minutes at 80°C (6.5 to 7.6-log reduction) and within two minutes at 90°C (6.0 to 7.7-log reduction). However, for *Salmonella* inoculated samples >5.60±0.6 log reductions was achieved at 90°C for four and five minutes. Other time and temperature combination for *Salmonella* and *E. coli* O157:H7 inactivation was unable to achieve a five-log reduction. The decimal reduction time (*D*-values) for *E. coli* O157:H7 isolates were lower than for *Salmonella* isolates. At, 70, 80, and 90°C the *D*-values for *E. coli* O157:H7 were 0.68, 0.52, and 0.46 minites, respectively, and for *Salmonella* were 1.02, 0.9, and 0.7 minutes, respectively.

Significance: Utilizing proper time and temperature of hot water treatment during pecan shelling could significantly reduce the food safety risk associated with *Salmonella* and *E. coli* O157:H7.

P3-42 Effectiveness of Hot Water and Peroxyacetic Acid Treatment on Inactivation of Inoculated *Salmonella* spp. on Alfalfa, Clover, and Radish Seeds Intended for Sprout Production

Namrata Karki[1], **KARUNA KHAREL**[1], Jorge Cabezas[2], Alejandro Arias[2] and Achyut Adhikari[1]
[1]Louisiana State University AgCenter, Baton Rouge, LA, [2]Zamorano University, Tegucigalpa, Honduras

Introduction: Fresh sprouts consumption for nutritional benefits has been a popular practice around the world. Regardless of the health promoting value, sprout consumption exposes the consumer to a high risk of microbiological contamination. The optimum environmental factors used for sprout production are also favorable conditions for the growth of pathogenic bacteria. *Salmonella* is one of the major pathogens associated with sprouts outbreak.

Purpose: This study investigated the effect of hot water (HW) and peroxyacetic acid (PAA) to inactivate *Salmonella* in alfalfa, clover, and radish seeds prior to germination.

Methods: Commercially purchased alfalfa, clover, and radish seeds were inoculated with a four serotype cocktail of *Salmonella enterica*. HW treatment (5 s) followed by PAA treatment at various concentration (30, 60, and 80 ppm) for different time intervals (2, 4, and 6 min) were applied to *Salmonella* inoculated seeds. Enumeration was performed using XLD agar. Data were analyzed using one-way ANOVA.

Results: Applied treatments of HW and PAA did not affect the rate of seed germination. Among three different types of seeds, these treatments were found to be the most effective in alfalfa seeds. HW alone was able to reduce three log CFU/g while additional treatment with PAA at 60 and 80 ppm for four and six minutes was able to reduce five log CFU/g in alfalfa seeds. However, for clover and radish seeds, treatment with HW and PAA resulted in an average microbial reduction of 3.67 log CFU/g and 1.87 log CFU/g, respectively, with no significant difference among the treatments. These results indicated that effect of PAA in combination with HW treatment is highly dependent on the type of seeds.

Significance: Our data suggested that sequential treatments of HW and PAA could be an effective measure in reducing microbial load in alfalfa seeds intended for sprout production.

P3-43 Assessing the Effectiveness of Sanitizer Spray and Brush Roller Treatment on Reducing the Population of a Five-strain *Salmonella enterica* Cocktail on Mango Surfaces

XINYUE WANG and Michelle D. Danyluk
University of Florida, Lake Alfred, FL

❖ ***Developing Scientist Competitor***

Introduction: Mangoes have been associated with at least five documented *Salmonella* outbreaks in North America. Sanitizer spray and brush roller treatment has been documented as an effective means of reducing *Salmonella* on the surface of produce.

Purpose: The purpose of this study was to evaluate the efficacy of chlorine, peroxyacetic acid (PAA), and chlorine dioxide (ClO_2) to reduce *Salmonella* populations on the surface of mangoes during brush treatment.

Methods: Tommy Atkins mangoes were spot inoculated with 100 µl five-strain *Salmonella* (rifampicin-resistant) cocktail (eight log CFU/mango) onto the equator and dried for one hour. Not inoculated (negative control) or inoculated mangoes were washed with a laboratory-scale brush roller system with either ground water (control), or sanitizers (100 ppm free chlorine (pH=7), 80 ppm PAA, or 5 ppm ClO_2) for 0, 5, 15, 30, and 60 s respectively (n=15). Dey/Engley buffer (100 ml) was added to whole mangoes in sterile bags to remove *Salmonella* from the surfaces using a rub-shake-rub method, then plated in duplicate onto selective and/or nonselective agar. Data were statistically analyzed by ANOVA and Tukey's HSD test for effect of sanitizer and time.

Results: Chlorine, PAA, and ClO_2 spray resulted in significantly higher (P<0.05) log CFU/mango reduction of *Salmonella* than water spray during brush roller treatment regardless of washing time. The highest log reduction with PAA treatment was at 60 s (5.07±1.05), compared with chlorine (4.43±0.91), ClO_2 (3.81±0.29), and water treatment (3.27±0.50). In all cases, log reductions increased with longer treatment times. ClO_2wash achieved at least 2.5 log reduction; chlorine and PAA wash achieved at least three-log and four-log reduction, respectively; while water wash alone achieved a 1.75-log reduction.

Significance: Addition of chlorine, PAA, or ClO_2 to spray water used during brush treatment can lead to more effective food safety management, which will help to minimize contaminated mangoes entering the market.

P3-44 Effect of Curli Expression and Adhesion of *Salmonella* Newport on Bacterial Transfer during Cucumber Peeling

JIIN JUNG[1] and Donald W. Schaffner[2]
[1]*Rutgers University, New Brunswick, NJ,* [2]*Rutgers University, Department of Food Science, New Brunswick, NJ*

❖ ***Developing Scientist Competitor***

Introduction: Fresh cucumbers have recently been recognized as a vehicle in foodborne disease outbreaks. Several United States multistate outbreaks of salmonellosis have been linked to fresh cucumbers.

Purpose: Little is known about microbial cell surface characteristics that affect adhesion on subsequent bacterial cross-contamination and transfer. This study determined the effects of surface characteristics of *Salmonella* on the transfer of *Salmonella* Newport from cucumber skin to interior flesh and peeler during peeling.

Methods: Wild type *Salmonella* Newport and putative attachment mutants, JDB 279 (*rpo*S:Tn*10*:lac:kan) and JDB 287 (Tn*10*:lac:kan insertion in the *agfD*/*agfB* intergenic region), were used to inoculate cucumbers. Bacterial transfer from cucumber skin to the flesh and peeler during peeling was quantified. The attachment strength values (S_R) of *Salmonella* Newport to the cucumber surface were calculated using an attachment assay method. The Congo red binding assay was used to determine curli expression of *Salmonella* Newport.

Results: The population of curli-positive *Salmonella* Newport wild type attached to the cucumber surface was greater than curli-negative mutant strains (JDB 279 and JDB 287) (P<0.0001). The S_R value of *Salmonella* Newport wild type on cucumber (0.59) was higher than that of JDB 287 (0.50) and JDB 279 (0.53), but not significantly different. Increased attachment of *Salmonella* Newport wild type on cucumber surface resulted in the lowest transfer to the flesh and peeler during cucumber peeling. Relatively higher bacterial transfer to the flesh (-0.84 log %, P=0.1346) and peeler (-0.60 log %, P=0.0277) was observed during peeling of cucumbers inoculated with JDB 287 compared to the transfer of *Salmonella* Newport wild type (-1.31 to -1.15 log %) and JDB 279 (-1.10 to -1.07 log %).

Significance: This study showed that *Salmonella* attachment does affect subsequent bacterial transfer during cucumber peeling. The findings will help in the development of washing and/or peeling technologies to reduce *Salmonella* contamination risk.

P3-45 Rapid Concentration/Detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes* from Lettuce Wash Waters Generated in Commercial Scale Facilities

RYANN GUSTAFSON[1], Sonia Magana[2], Elizabeth Kearns[2], Daniel Lim[2] and Elliot Ryser[1]
[1]*Michigan State University, East Lansing, MI,* [2]*University of South Florida, Tampa, FL*

❖ ***Developing Scientist Competitor***

Introduction: A dead end ultrafiltration concentration (DEUF-C) sampling method, previously demonstrated, increased detection probability of pathogens in lettuce wash water (LWW) in pilot-scale testing.

Purpose: This study investigated use of DEUF-C in commercial-scale tests through the following two objectives: 1) comparison of pathogen detection probabilities using DEUF-C versus standard grab sampling in LWWs generated from a processing test line and 2) assessment of DEUF-C to concentrate pathogens spiked into commercially generated flume and centrifugation LWWs.

Methods: In objective 1, 2 to 4×10^4 CFU of *Escherichia coli* O157:H7 (*Ec*) and 3 to 9×10^5 CFU of *Listeria monocytogenes* (*Lm*) were spiked into one lettuce head and processed along with 907 kg of lettuce and sanitizer-free tap water. Two to four 40 liter volumes of LWW were processed by DEUF-C to generate 400 ml concentrated samples. Detection of probabilities for DEUF-C samples were compared to standard grab samples after 24 h enrichment/qPCR. In objective 2, flume and centrifuge LWW were collected from a commercial plant, treated with sodium thiosulfate (100 mg/liter) to neutralize the chlorine-based sanitizer, spiked with both *Ec* and *Lm* at ~10^2 CFU/ml and, then, processed by DEUF-C to determine total filterable volumes (TFVs). Detection (qPCR) probabilities in nonenriched DEUF-C and grab samples were determined to compare target organism recoveries. Experiments were repeated in quadruplicate.

Results: In objective 1, *Ec* and *Lm* were detected in 100% of DEUF-C samples (n=14) compared to 6.7% and 20%, respectively, in standard grab samples (n=15). In objective 2, TFVs for flume water were higher (n=4; 30.6+9.9 L) than those for centrifugation water (n=4; 9.9+2.0 L) (P=0.029, Mann-Whitney U Test). *Ec* and *Lm* were detected in 100% of DEUF-C samples compared to 11.1% and 16.7%, respectively, in standard grab samples.

Poster

Significance: DEUF-C offers improved probability of detection for *E. coli* and *Listeria*; however, TFV for commercial produce wash water needs to be further improved.

P3-46 Prevalence, Persistence, and Transfer of Antimicrobial-resistant Microorganisms from Organic Dairy Manure to Leafy Greens

NITYA SARJAPURAM, Maria Albarracin, Siddhartha Thakur and Eduardo Gutierrez
North Carolina State University, Raleigh, NC

Introduction: Antimicrobial resistant microorganisms have been recognized as a serious threat to public health, globally. Transfer of antimicrobial resistance (AMR) within our food supply and healthcare systems has severely impacted our ability to treat and prevent disease. General distribution of AMR in produce fields could pose as a potential human health risk.

Purpose: To evaluate the presence, persistence, and transfer of AMR *Enterobacteriaceae* bacteria from organic dairy manure to organically grown spinach plants.

Methods: The presence of 3rd generation cephalosporin-resistant *Enterobacteriaceae* was assessed from organic dairy cow manure, soil, spinach plants, and soil-manure-spinach mesocosms. *Enterobacteriaceae*, *Escherichia coli*, and Shiga toxin producing *E. coli* (STEC) were extracted and plated on selective and differential media supplemented with four different ceftiofur concentrations (0 µg/ml (control), 4 µg/ml (susceptible), 8 µg/ml (intermediate), and 16 µg/ml (resistant)). Bacterial recovery was performed at 0, 7, 14, and 30 days from six independent replicates each of 150 g. A subset of 65 *Enterobacteriaceae, E. coli,* and STEC bacteria were further screened for multidrug resistance (MDR) to a panel of 15 antimicrobials using the broth microdilution method.

Results: Significant differences (P<0.05) in the population of ceftiofur susceptible and resistant *Enterobacteriaceae* were observed in organic dairy cow manure (4.2 and 1.2 log CFU/g, respectively). The entire STEC population showed ceftiofur resistance, while no differences were observed between antibiotic concentrations. In soil-manure-spinach mesocosms, no significant differences (P<0.05) in the *Enterobacteriaceae* population were observed over 30 days, while for *Eschericha coli* no detectable resistant populations were determined after 14 days of initiating each experiment. Further screening of MDR from ceftiofur-resistant and nonresistant *Enterobacteriaceae* and STEC, indicated a high incidence of antibiotic resistance to sulfisoxazole (72%), streptomycin (55%), and tetracycline (52%).

Significance: Under the selected conditions (15-year-old organic system) there was wide spread distribution of MDR *Enterobacteriaceae,* which could pose a threat to human health.

P3-47 Visualizing Pathogen Internalization Pathways in Fresh Tomatoes Using a Confocal Laser Scanning Microscope and a Micro CT

BIN ZHOU[1], Yaguang Luo[1], Gary Bauchan[1], Hao Feng[2] and John Stommel[1]
[1]U.S. Department of Agriculture–ARS, Beltsville, MD, [2]University of Illinois, Urbana, MD

Introduction: Pathogen contamination and internalization in fresh produce significantly impacts public heath and the industry's economic well-being. In tomato fruit, studies have shown that stem scars play an important role in pathogen infiltration. However, the exact mechanisms and pathways for pathogen movement inside tomatoes are unclear.

Purpose: This study examined in detail the mechanisms of pathogen internalization in tomatoes.

Methods: Tomato fruit infused with potassium iodine and examined with Xradia Bio MicroCT at 0.5x ~ 4x revealed that infiltrated potassium iodine moved along the vascular bundles inside the fruit. We assessed whether or not vascular tissues were a similar means for pathogenic *Escherichia coli* infiltration. Tomato fruit were infiltrated with red fluorescent microspheres and *E. coli* O157:H7 - pGFP. Stem scars and core tissues were subsequently excised after fruit surface disinfection. Samples were observed using a Zeiss 710 confocal laser scanning microscope, or stomached, cultured, and enumerated for inoculated *E. coli* O157:H7.

Results: Populations of internalized *E. coli* O157:H7 were confined in xylem vessels and concentrated immediately beneath the stem scar, with a sharp decline in population with vertical distance from the stem scar. Our observations suggest that 1) vascular bundles, especially the xylem vessels in tomatoes, play a critical role in pathogen internalization; and 2) pathogen internalization is a passive, rather than active process, as the infiltration and movement of both microspheres (nonliving) and *E. coli* O157:H7 cells (living) behaved similarly inside tomatoes.

Significance: This study presents the first visual evidence of the critical role of vascular bundles, in pathogen internalization. The information generated is important for developing science-based food safety practices and interventions in controlling pathogen internalization and may be useful for future breeding effort in developing pathogen internalization resistant varieties.

P3-48 Under Field Conditions, Distance is Significantly Associated with the Amount of *Escherichia coli* That Transfers from Wildlife Feces to Preharvest Lettuce during Foliar Irrigation

DANIEL WELLER, Jasna Kovac, David Kent, Sherry Roof, Jeffrey Tokman and Martin Wiedmann
Cornell University, Ithaca, NY

❖ ***Developing Scientist Competitor***

Introduction: Previous studies have (i) found that splash during rain or irrigation events can transfer microbes from the surrounding environment (e.g., wildlife feces) to preharvest produce, and (ii) identified wildlife as potential vectors for the introduction of foodborne pathogens into farm environments. However, only two peer-reviewed studies have examined the transfer of pathogens from wildlife feces to field-grown produce. While both studies found a significant relationship between distance and *Escherichia coli* transfer, the studies only sampled produce that was close to the feces (e.g., <1 m).

Purpose: This study was conducted to provide additional data to refine our understanding of the impact of distance on *E. coli* splash.

Methods: Two trials were conducted; for each trial rabbit feces inoculated with a three-strain cocktail of nonpathogenic *E. coli* were placed in a lettuce field 2.5 hours before irrigation. Following irrigation, the *E. coli* concentration on lettuce, between 0.0 and 5.2 m from the feces, was determined. For analysis, data were combined with a subset of data from a previously published study.

Results: Thirty nine percent (38 of 97) lettuce heads had detectable levels (≥1 MPN) of *E. coli*. While none of the 39 lettuce heads ≥2 m from the closest feces had detectable levels of *E. coli*, 38 of 58 heads that were <2 m from the feces tested positive for *E. coli.* Linear regression showed that, for lettuce <2 m from the feces, the *E. coli* concentration decreased by 1.78 (95% Confidence Interval=-2.52, -1.05; P<0.0001) log MPN for each meter increase in the lettuce-feces distance.

Poster

Significance: Establishing a no-harvest buffer around in-field feces may reduce the risk of harvesting contaminated produce. As such, this study provided data that can be used in quantitative risk assessments to develop effective risk management strategies following wildlife intrusion.

P3-49 Survival of Generic *Escherichia coli* and *Listeria* spp. Populations in Dairy Compost- and Poultry Litter Compost-amended Soils in the Northeastern United States

MARIE LIMOGES[1], Deborah Neher[1], Thomas Weicht[1], Patricia Millner[2], Manan Sharma[2] and Catherine Donnelly[1]

[1]University of Vermont, Burlington, VT, [2]U.S. Department of Agriculture–ARS, Beltsville, MD

Introduction: The FDA FSMA standards stipulate composting conditions that meet acceptable treatments for use of manure/poultry litter-based biological soil amendments of animal origin (BSAAO). Application of FSMA-compliant BSAAO to soils for production of fresh produce is expected to result in reduced risk of pathogen contamination on the harvested produce when other stipulations in the Produce Safety rule are also implemented. However, meteorological conditions, geographic location, application methods, soil type, and bacterial populations can influence the presence of pathogenic bacteria, or their indicators (e.g., generic *E. coli*) and potential produce contamination.

Purpose: Evaluate survival of nonpathogenic *E. coli* (*gEc*) and *Listeria* spp.in tilled plots with both dairy and poultry litter composts in the northeastern United States.

Methods: Replicated field plots (2m^2, *n*=24) of Loamy (L) or Sandy (S) soils were tilled and amended with dairy compost (DC), poultry litter compost (PLC), or no compost (NC). These plots were inoculated with a three-strain cocktail of rifampicin-resistant *gEc* at a rate of 8.7 log CFU/m^2. Colony count and most probable number (MPN) methods were used to determine persistence of *gEc* in these plots over 104 days post-inoculation (dpi). Detection of indigenous *Listeria* spp. were also examined in all plots.

Results: At 104 dpi, inoculated *gEc.*survived at higher populations in PLC plots (3.5 to 3.80 log MPN/gdw) in comparison to DC plots (0.06 to 3.8 log MPN/gdw) and NC plots (0.6 to 1.3 log MPN/gdw). Populations of gEc and native *E. coli* after rainfall were independent of soil type. *Listeria* spp. were found in PLC and DC, but not in NC plots.

Significance: Results are consistent with those from studies conducted in other regions of the United States that show that poultry litter-based BSAAO support greater numbers and longer periods of persistence in field soils of *gEc* than dairy-based BSAAO.

P3-50 The Effect of Soil Management on the Persistence of *Escherichia coli* and *Listeria* spp. in Manure-amended Soils in the Northeast United States

PANAGIOTIS LEKKAS[1], Manan Sharma[2], Deborah Neher[1], Thomas Weicht[1], Patricia Millner[2] and Catherine Donnelly[1]

[1]University of Vermont, Burlington, VT, [2]U.S. Department of Agriculture–ARS, Beltsville, MD

Introduction: Enterohemorrhagic *Escherichia coli* and *Listeria monocytogenes* can contaminate leafy greens through inappropriately managed raw manure applied to soils. Current FDA guidance includes calling for additional scientific data to determine the appropriate interval between application of manure and harvest of crops grown in manure-amended soils to minimize risk of human illness.

Purpose: To determine persistence of *E. coli* and *Listeria* spp. populations in sandy and loamy soils amended with dairy-manure solids (DS) in Vermont.

Methods: One liter of a three-strain inoculum (TVS353,TVS354, and TVS355) of rifampicin-resistant *E. coli* (*rEc*) (six log CFU/ml) was sprayed onto field plots (2m^2) of either loamy sand (L) or sandy (S) soils amended with DS or no manure (NM). DS was either tilled into soil or spread on the surface. Survival of inoculated *rEc*, naturally present *E. coli* (*nEC*) and *Listeria* spp. in DS in untilled and tilled plots were quantified over 56 days postinoculation (dpi) by direct plating or MPN.

Results: By 56 dpi, in surface plots, *rEc* populations declined by 3.65 to 3.69 log CFU/g and 3.05 to 3.11 log CFU/g in L and S soils, respectively. In tilled plots, *rEc* populations declined by 2.27 to 2.33 and 2.08 to 2.14 log CFU/g in L and S soils, respectively. In L and S soils, *rEc* populations in surface NM declined to undetectable levels by 14 and 28 dpi, respectively. Overall, *Listeria* spp. populations increased by 0.05 to 0.96 log MPN/g by day 56, and *Listeria spp*. were present in both NM and DM-amended soils. The *nEc*populations declined by 1.15 to 1.60 log CFU/g by 56 dpi.

Significance: In comparison to studies conducted in the two previous years at the same sites, *rEc* populations declined more rapidly by seven dpi in this iteration. Results presented from this year's study indicates that soil tillage influences survival of *E. coli* as much as dairy manure amendment.

P3-51 Low Prevalence of Foodborne Pathogens Found in Produce Grown on Diversified Farms in California

Nora Navarro-Gonzalez[1], Laura Patterson[2], Peiman Aminabadi[1], Alda Pires[2], Shirley Micallef[3], Robert Buchanan[4] and **MICHELE JAY-RUSSELL**[1]

[1]University of California-Davis, Western Center for Food Safety, Davis, CA, [2]University of California-Davis, Department of Population Health & Reproduction, Davis, CA, [3]University of Maryland, College Park, MD, [4]University of Maryland, Department of Nutrition and Food Science and Center for Food Safety and Security Systems, College Park, MD

Introduction: Diversified farms raise a wide range of animal and plant varieties, and are usually small to medium-sized with direct sales to consumers. Their popularity and importance is increasing, but practices associated with this type of farming system may pose a risk for food safety.

Purpose: To determine the prevalence of foodborne pathogens, *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC), in produce from California diversified farms, and to quantify bacterial indicators of fecal contamination.

Methods: Three hundred sixty-seven leafy green and 305 tomato samples were collected from 17 diversified farms from 2015 to 2016. Generalized linear models were conducted to evaluate the association between *E. coli* prevalence and produce type, collection type, farm status, environment, irrigation water type, and period between manure application and harvest. The best model was selected based on Akaike's information criterion.

Results: No sample was positive for *Salmonella* or *E. coli* O157:H7. One targeted spinach sample was positive for STEC O136. Random (11.6%; *n*=198) and targeted leafy greens (11.8%; *n*=169) were positive for generic *E. coli* (median=0 MPN/100 g, range 0 to 1.2×10^7 MPN/100 g). The prevalence of fecal coliforms in random and targeted leafy greens was 31.3% and 37.3%, respectively (median=0 MPN/100 g, range 0 to 1.4×10^8 MPN/100 g). For tomatoes, 4.9% of random samples (*n*=163) and 6.3% of targeted samples (*n*=142) were positive for generic *E. coli* (median=0 MPN/100g, range 0 to 4.6×10^5 MPN/100 g). Fecal coliforms were detected in 68.7% and 75.2% of tomato samples (median=1.2×10^3 MPN/100g, range 0 to 2.8×10^9 MPN/100 g). Noncertified organic samples had a higher probability of being *E. coli* positive. For total fecal coliforms, the best model included the use of surface water.

Significance: The prevalence of foodborne pathogens found in produce on diversified farms is low. Noncertified organic farm status and the use of surface water were identified as potential risk factors for fecal contamination of produce.

Poster

P3-52 Microbial Attachment and Die-off Rate on Watermelon Surface in an Agricultural Setting

VIJAY SINGH CHHETRI, Kathryn Fontenot, Ronald Strahan, Karuna Kharel, Namrata Karki and Achyut Adhikari
Louisiana State University AgCenter, Baton Rouge, LA

❖ ***Developing Scientist Competitor***

Introduction: Preharvest environmental condition may influence the attachment of microorganisms on produce surface.

Purpose: This study examined the attachment and die-off rate of microorganisms on watermelon surface in an agricultural setting.

Methods: Replicated watermelon plots (18 plots, three plots per treatment, plot size 12' by 30', 30 plants per plot) were treated with five varieties of herbicides that resulted in different weed levels in each plot. At the time of harvesting, a cocktail of generic *E. coli* (ATCC 23716, 25922, and 11775) was inoculated on the outer surface (around 50 cm^2) of the watermelon fruits (*n*=162). The attachment strength (SR) and die-off rate of generic *E. coli* was examined up to 120 hours. Samples (0 and 120 hours) were treated with a chlorine solution (150 ppm) to evaluate the effect of sanitizer against the firmly and loosely attached generic *E. coli*.

Results: The attachment strength (SR value) and die-off rate of inoculated generic *E. coli* on watermelon surface increased significantly ($P<0.05$) within 24 hours. The initial SR value of 0.04 reached 0.99 after 120 h. There was a significant increase in firmly attached generic *E. coli* levels (from 3.93 log CFU/cm^2 to 4.47 log CFU/cm^2) after 72 h; however, within 24 h the loosely attached bacteria reduced to at or below the detectable limit of the test. After 96 h, the total generic *E. coli* levels on the watermelon surface was reduced 5.68 log CFU/cm^2. The chlorine treatment resulted in the reduction of generic *E. coli* level by 4.22 log CFU/cm^2 and 0.61 log CFU/cm^2on the 0 h and 120 h samples, respectively.

Significance: The finding suggests that preharvest environmental conditions can induce microbial attachment and biofilm formation on produce surface which may affect subsequent postharvest processing and sanitizing treatments.

P3-53 Effect of Residual Chlorine on the Attachment and Survival of *Escherichia coli* O157:H7 on Spinach Surface

VIJAY SINGH CHHETRI and Achyut Adhikari
Louisiana State University AgCenter, Baton Rouge, LA

❖ ***Developing Scientist Competitor***

Introduction: Recent foodborne disease outbreaks highlighted produce packing environment as an important source of contamination. After sanitization, produce are exposed to the environment for several minutes before packaging, which may increase the risk of cross-contamination.

Purpose: This study examined the residual chlorine level on spinach after sanitization and its effect on the attachment and die-off rate of *Escherichia coli* O157:H7 on the surface of the leaf.

Methods: Baby Spinach was purchased from a local retail store. The intact leaves with similar size and appearance were treated with chlorine solution (150 ppm) for three minutes. The residual chlorine level on spinach surface after the treatment was examined up to 90 minutes using DPD Colorimetric Method. A cocktail of *E. coli* O157:H7 (H1730, ATCC 43895, EC 4042) was inoculated on the surface (8 cm^2) of the leaves immediately and 30 minutes after treatment with chlorine. The effect of residual chlorine on the attachment and die-off rate of *E.coli* O157:H7 was examined up to 48 hours.

Results: A rapid decline in both free chlorine (7.2 to 0.3 mg/kg) and total chlorine (10.6 to 1.5mg/kg) level was observed on spinach surface treated with chlorine within 90 minutes of storage at 4°C. The residual chlorine present in freshly treated and 30 minutes after chlorine treated samples significantly ($P<0.05$) reduced the *E.coli* O157:H7 level on spinach surface. The reduction was by 1.30 log CFU/cm^2 on the freshly treated samples and by 1.14 log CFU/cm^2 on 30 minutes after treated samples. Further reduction (2.78 log CFU/cm^2) was observed on freshly treated samples after 24 hours of storage. The SR values which indicate the attachment strength of microorganisms on produce were significantly higher on chlorine treated samples than on untreated samples.

Significance: Our study suggests that the residual chlorine on produce surfaces may minimize the survival of postsanitation microbial contamination.

P3-54 Interactions between *Salmonella enterica* Newport and Plant Pathogenic Fungi of the Genus *Fusarium* on Melons

ROBERT KORIR[1], Shirley Micallef[2] and Kathryne Everts[2]
[1]*University of Maryland College Park, Salisbury, MD,* [2]*University of Maryland, College Park, MD*

❖ ***Developing Scientist Competitor***

Introduction: Several *Salmonella* outbreaks have occurred on melon fruit over the past few years.

Purpose: To investigate the relationship between plant pathogenic *Fusarium* spp., which cause fruit rot on melon, and *Salmonella enterica* Newport. The survival and growth of *Salmonella* Newport on five different melon cultivars following inoculation of *Fusarium* spp. was evaluated.

Methods: Melon rind disks (A=38.5cm^2) of the cultivars Arava, Athena, Dulce, Juane and Sivan were inoculated separately with three *Fusarium* spp. (100 µl- 4 log spore ml^{-1}) or water and incubated. The treatments were 1) no *Fusarium* infection (control), and *Fusarium* infection with 2) *Fusarium oxysporum*, 3) *Fusarium fujikuroi*, 4) *Fusarium armeanicum.* Four days later, *Fusarium* infection was followed by inoculation with 100 µl (~10^4 CFU ml^{-1}) *Salmonella* Newport, adapted for rifampicin resistance, and incubated at 25°C for 24 h. The experiment was repeated three times (*n*=15/cultivar). *Salmonella* Newport was recovered in 0.1% buffered peptone water, plated onto tryptic soy agar with rifampicin and cycloheximide (50µg ml^{-1} each), and incubated at 37°C for 24 h. Counts in log CFU ml^{-1}were analyzed using mixed model (JMP).

Results: *Salmonella* Newport was not recovered from *Fusarium*-infected only or water only treatments. *Fusarium* spp. infection of melons had no significant impact on *Salmonella* Newport populations. However, less *Salmonella* Newport ($P<0.0001$) was recovered from the non-netted cultivars, Dulce (3.9, 4.2, and 4.0 log CFU/ml) and Juane (3.5, 3.7, and 3.1 log CFU/ml) compared to the netted, Arava (5.7, 5.6, and 5.7 log CFU/ml), Athena (5.6, 7.0, and 6.2 log CFU/ml), and Sivan (4.5, 5.6, and 5.9 log CFU/ml) when inoculated with *F. oxysporum, F. armeanicum* and water control, respectively.

Significance: This study provides evidence that *Fusarium* fruit infection of a variety of melon cultivars does not affect *Salmonella* melon surface colonization. *Salmonella* Newport survival and colonization on melons, however differs among cultivars.

P3-55 Evaluating the Recovery of *Salmonella* from Enriched Inshell Walnuts

ADAM COBERT and Linda J. Harris
University of California-Davis, Davis, CA

❖ ***Developing Scientist Competitor***

Introduction: The prevalence of *Salmonella* on in shell walnuts, as determined by enrichment in buffered peptone water (BPW) and detection with the mini-VIDAS system, was significantly lower than reported for other tree nuts.

Purpose: The objective of the study was to evaluate the impact of in shell walnuts in the recovery of inoculated *Salmonella* using enrichment and the mini-VIDAS detection method.

Methods: Inshell walnuts were collected from three collaborating hullers shortly after harvest in two separate years (*n*=6 lots). Approximately 50 CFU of rifampicin-resistant *Salmonella* Enteritidis PT 30 was inoculated into 450 ml of BPW with and without three inshell walnuts (~45 g), and was incubated at 42°C for 18 or 24 h. Samples were spiral-plated onto selective agar to determine levels of *Salmonella* and, in some cases, processed with the mini-VIDAS system.

Results: The limit of detection (LOD) for *Salmonella* by the mini-VIDAS was five or six log CFU/ml in the absence or presence of walnuts, respectively. After incubation, background population levels of uninoculated walnuts consistently reached greater than eight log CFU/ml. Levels of *Salmonella* in BPW controls without walnuts were consistently greater than seven log CFU/ml. When walnuts were present, levels of *Salmonella* ranged from below the LOD of plating (0.6 log CFU/ml) to >7 log CFU/ml after 18 to 24 h at 42°C. Significant sample, huller, and year effects were observed. Four of 87 samples were below the LOD by plating and an additional 12 of the 87 samples were below the LOD for the mini-VIDAS (<6 log CFU/ml).

Significance: The presence of inshell walnuts can inhibit the growth of *Salmonella* during enrichment, which could lead to an inability to detect *Salmonella* by methods that rely on high concentrations of the target pathogen.

P3-56 *Salmonella* Serovar Specific Tolerance to Nitric Oxide Stress In Vitro and in the Tomato Phyllosphere

ANGELA FERELLI and Shirley Micallef
University of Maryland, College Park, MD

❖ ***Developing Scientist Competitor***

Introduction: Not much is known about the role of nitric oxide (NO), a key plant immune signaling molecule, in the *Salmonella*-tomato interaction.

Purpose: This study investigated whether *Salmonella* is affected by plant-derived NO when colonizing plant surfaces and evaluated *Salmonella* serovar-specific responses to NO stress in vitro and in the tomato phyllosphere.

Methods: To determine whether NO on tomato affects *Salmonella* colonization, 0.2 mM of the NO scavenger 2-4-carboxyphenyl-4, 4, 5, 5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) was applied via spray to five-leaf stage cv. Heinz seedlings that were subsequently inoculated with five log CFU/mL of *Salmonella* Newport, Javiana, or Typhimurium. Culturable cell counts via direct plating were measured at 12 and 24 hours postinoculation (hpi). To investigate the bactericidal capacity of free NO in solution, *Salmonella* Javiana, Newport, Enteritidis, Heidelberg, or Typhimurium were exposed to various concentrations (125 µM to 2mM) of the NO donor spermine NONOate in phosphate buffered saline. Cell counts via dilution plating were taken at 1, 4, and 24 hpi. Statistical analysis included ANOVA and Tukey-HSD.

Results: *Salmonella* on tomato leaves showed significantly positive population responses to NO scavenging (P<0.05) in *Salmonella* Javiana and *Salmonella* Newport, but not *Salmonella* Typhimurium samples. In vitro, evaluating of the log decline from inoculum at each NO concentration revealed that one hpi exhibited the highest degree of disparity in serovar culturability (P<0.05). Furthermore, *Salmonella* Heidelberg more often displayed significantly larger log declines than other serovars tested (P<0.05). Conversely, *Salmonella* Newport and Javiana had smaller log declines relative to other serovars tested (P<0.05).

Significance: *Salmonella* Newport and *Salmonella* Javiana responded positively to the removal of NO on leaf tissue, providing evidence that plant responses to *Salmonella* are able to restrict bacterial populations. These two serovars also exhibited smaller log declines in response to NO stress. Produce outbreak-associated serovars, such as *Salmonella* Newport and Javiana, may have adaptations that aid survival in the phyllosphere.

P3-57 Water Stress Limits the Growth of *Salmonella* on the Lettuce Leaf Surface

XINGCHEN LIU and Shirley Micallef
University of Maryland, College Park, MD

Introduction: *Salmonella enterica* can survive on lettuce leaf surfaces using nutrients from lettuce exudates. Studies have reported that polyphenols are negatively correlated with *Salmonella enterica* growth. When plants experience water stress, as occurs in hot weather, during dry spells, and in between irrigation, they are likely to release more polyphenols.

Purpose: The purpose of this study was to evaluate whether water stress experienced by lettuce plants would impact *Salmonella enterica* associated with lettuce leaf surfaces.

Methods: Four-week-old romaine lettuce plants (cv. Parris Island Cos) grown in a greenhouse (16 hours light at 26°C, 8 hours dark at 18°C) were subjected to water stress for four days by withholding irrigation. Control plants were watered regularly. Cultures of *Salmonella* Typhimurium, adapted for rifampicin resistance, grown overnight on tryptic soy agar (TSA) amended with 50 µg/ml rifampicin at 35°C, were resuspended in sterile water to 10^6 CFU/ml. Ten drops (total 100 µl) of the cell suspension were spot-inoculated on the abaxial side of each leaf. After 24 hours, the inoculated leaves were clipped, and placed in a whirlpak bag with 30 ml of 0.1% peptone water. After shaking at 250 rpm for 10 min, serial dilutions were prepared and plated onto TSA with 50 µg/ml rifampicin for bacterial enumeration.

Results: There were statistically significant differences (P<0.05) in the recovered counts of *Salmonella* Typhimurium between water-stressed plants and control plants. When leaves were inoculated with ~7.6 log CFU *Salmonella* Typhimurium/ml, the population recovered from water-stressed plants decreased to an average of 1.9±0.5 log CFU/ml. On control plants, the average population was measured at 2.8±9.4 log CFU/ml.

Significance: These data suggested that lettuce plants that are responding to water stress may be less favorable for epiphytic *Salmonella*.

P3-58 Survival of *Salmonella* spp. and *Listeria monocytogenes* on Pressed Card and Plastic Polyethethylene Board from the Farmers' Market and Validated Commercial Sanitizers to Decontaminate *Salmonella* spp. and *Listeria monocytogenes*

KA WANG LI, Lacey Lemonakis and Cangliang Shen
West Virginia University, Morgantown, WV

❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* and *Listeria* are present on fresh produce at farmers' markets. Uncleaned containers could be a potential contamination source.

Purpose: This study evaluated the potential differences in survival of *Salmonella* and *Listeria monocytogenes* (*Lm*) on pressed-card and plastic polyethethylene surfaces during storage and evaluated antimicrobial efficacy of commercial sanitizers.

Methods: Study I, an anonymous survey, assessed postharvest practices of handling storage containers from 28 vendors at the Morgantown, WV, farmers' market. In Study II, pressed-card (green moulded-pulp produce basket) and plastic containers, obtained from the farmers' market, were trimmed (4 cm by 4 cm) and inoculated with *Salmonella* Typhimurium and Tennessee and three strains of *Lm*, stored at 4°C and 25°C (22 days), and periodically analyzed for survivals of inoculated microorganisms. Study III, evaluated pressed-card and plastic board inoculated with *Salmonella* and *Lm*, stored at 4°C for 1 and 24 h, treated by immersion into tap water, sodium hypochlorite (200 ppm), lactic acid (5%), peroxyacetic acid (PAA, 1,000 ppm), and H_2O_2-PAA mix (SaniDate-5.0, 0.25%) for 60 s. Surviving bacteria were analyzed on XLT-4 (*Salmonella*) and Modified-Oxford (*Lm*) agar. Data (two replicates/three samples/replicate) were analyzed using the mixed-model procedure of SAS and USDA Integrated Predictive Modeling Program software.

Results: Plastic (81%) and paper baskets (52%) were used by vendors. Forty-one percent of vendors did not sanitize containers. The pathogens died slower (P<0.05) at 4°C and on pressed-card (fit Reparamerized Gompertz survival model) than at 25°C and on plastic board (fit linear model without tail). At 4°C, *Salmonella* decreased from 4.2 to 4.4 to 3.1 to 3.2 log CFU/cm^2; *Lm* decreased from 6.3 to 6.4 to 3.9 to 4.2 log CFU/cm^2 during 22-day storage on both surfaces. At 25°C, pathogens decreased from 4.5 to 6.2 log CFU/cm^2 to <0.3 log CFU/cm^2 after two to four days on plastic board, which is shorter (P<0.05) than pressed-card (22 days). At 1 and 24 h, all sanitizers reduced *Salmonella* and *Lm* to <0.3 log CFU/cm^2.

Significance: Farmers' market vendors should choose plastic container to store fresh produce and avoid putting the containers in a cooler.

P3-59 The Efficacy of Chlorine and Acidified Sodium Chlorite for Disinfection of Seeds Artificially Inoculated with *Salmonella* as Affected by Treatment Time, Concentration, and Seed Type

ZIJING ZHANG[1], Yingqun Lei[1], Mengqi Li[1] and Tong-Jen Fu[2]
[1]Illinois Institute of Technology, Institute for Food Safety and Health, Bedford Park, IL, [2]U.S. Food and Drug Administration, Division of Food Processing Science and Technology, Bedford Park, IL

❖ ***Developing Scientist Competitor***

Introduction: Treatment of seeds with 20,000 ppm calcium hypochlorite, $Ca(OCl)_2$, is considered the gold standard; however, the high level of chlorine is potentially hazardous. There is a need to determine whether prolonged treatment with lower levels of chlorine would achieve similar degrees of pathogen reduction. Certain organic sanitizers (e.g., acidified sodium chlorite) exhibited equivalent or better efficacy in treating alfalfa seeds. Whether similar performance will be observed in other types of seeds remains to be determined.

Purpose: The efficacy of chlorine (prepared as $Ca(OCl)_2$) and acidified sodium chlorite (ASC) for treatment of six types of seeds/beans (alfalfa, clover, broccoli, onion, radish, and mung beans) artificially inoculated with *Salmonella* and the impact on germination rates, as affected by treatment time, concentration, and seed type was evaluated.

Methods: Ten grams seeds inoculated with four to six log cfu/g of *Salmonella* Typhimurium expressing green fluorescent protein were treated with different concentrations of $Ca(OCl)_2$ or ASC (200 to 20,000 ppm or 200 to 1,500 ppm, respectively) for different times (0.25, 0.5, 1, 3, 6, 16, or 24 h). After treatment, seeds were rinsed twice with sterile water and analyzed for *Salmonella* by plate counts and culture enrichment.

Results: Treatment with 20,000 ppm $Ca(OCl)_2$ for 15 min resulted in a greater log kill for clover, onion, and mung beans (reductions of 2.8, 3.1, and 3.8 log units, respectively) than for alfalfa seeds (1.3 log units). Free chlorine was rapidly consumed during treatment (from 2,000 ppm to < 0.02 ppm after 3 h). The efficacy of ASC increased with increasing concentration and treatment time. Treatment of clover, broccoli, and mung beans with 1,500 ppm of ASC for 3 h reduced *Salmonella* levels by 2.6, 4.2, and 3.6 log units, respectively, to undetectable (<1 log cfu/g).

Significance: Longer treatment with lower concentrations of chlorine was not effective due to the rapid disappearance of free chlorine. Acidified sodium chlorite provided an effective seed treatment option.

P3-60 A Blend of Benzalkonium Chloride, Acetic Acid, and Methyl Paraben Effectively Reduces *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* in Produce Wash Water, But Lacks Consistent Efficacy on Romaine and Iceberg Lettuce

NICHOLAS SEVART[1], Sara Gragg[2], Matthew Krug[1], Jacob Jenott[2] and Randall Phebus[1]
[1]Kansas State University, Manhattan, KS, [2]Kansas State University, Olathe, KS

Introduction: Illnesses associated with fresh produce demonstrates the need for effective intervention techniques. Improving the sanitizing efficacy of postharvest washes is a critical goal for improving fresh produce safety; however, contamination is difficult to address in fresh-cut produce such as lettuce.

Purpose: Efficacy of a proprietary blend of benzalkonium chloride, acetic acid and methyl paraben (BAM) was evaluated as a wash to reduce *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* O157:H7 on fresh-cut romaine and iceberg lettuce. Products were subjected to concentration/time combinations of the BAM wash and pathogen survival on product and resultant wash water was determined.

Methods: Freshly chopped romaine and iceberg lettuce was mist inoculated with *L. monocytogenes*, *Salmonella*, or *E. coli* O157:H7 (~ seven log CFU/g) and allowed to attach for 30 minutes. Lettuce was submerged with agitation in a 0, 1, 2 or 3% wash for one or five minutes, rinsed for one minute in tap water, and dewatered. Lettuce and wash water samples were homogenized in D/E Neutralizing Broth, and serial dilutions were spread plated using an injury-recovery agar overlay protocol to enumerate surviving *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 levels.

Results: All BAM-containing wash waters harbored less (P≤0.05) pathogenic contamination than the 0% control treatment. Concentration, contact time, and their interaction were not significant (P>0.05) for all pathogens on iceberg or romaine, with the exception of concentration (P=0.0021) for *E. coli* O157:H7-inoculated romaine. The 3% wash removed 0.66 log CFU/g more *E. coli* O157:H7 than 0% on the romaine lettuce.

Significance: Controlling pathogenic contamination in postharvest produce wash water is critical for preventing cross-contamination during commercial washing operations. The proprietary BAM wash was effective as a wash water sanitizing agent. Because pathogen reduction was lacking on fresh-cut lettuce, likely due to internalization of the inoculum within the lettuce tissue during washing, future studies should investigate efficacy on other products.

P3-61 Transfer of Microorganisms from a Dairy Calf Operation to an Adjacent Pistachio Orchard

CHRISTOPHER THEOFEL[1], Thomas Williams[1], Eduardo Gutierrez[2], Gordon Davidson[3], Michele Jay-Russell[4] and Linda J. Harris[1]

[1]University of California-Davis, Davis, CA, [2]North Carolina State University, Raleigh, NC, [3]U.S. Food and Drug Administration, College Park, MD, [4]University of California-Davis, Western Center for Food Safety, Davis, CA

Introduction: In recent surveys, *Salmonella* has been isolated from raw unprocessed pistachios at a prevalence of about 0.60%. Sources of *Salmonella* in pistachios are not well understood including the potential for contamination in the orchard prior to or during harvest.

Purpose: To evaluate transfer of microorganisms from a livestock operation to an adjacent pistachio orchard.

Methods: Orchard surface (drag swab), air (microbiological air sampling device), soil, and dust (pistachio leaf surface) samples were collected in an orchard adjacent (<20 m distance) to a commercial dairy calf operation (livestock orchard) and a single control orchard (surrounded by other pistachio orchards) over a two-year period. Air and cattle manure samples were collected within the livestock operation. Dry solids rinsed from leaf surfaces, aerobic plate count, presence of *Salmonella* and *Escherichia coli,*and bacterial community analysis through 16S rRNA sequencing were determined.

Results: *Salmonella* was isolated from manure and from 4 of 63 drag swabs collected from within the livestock orchard, but not from drag swabs collected within the control orchard (0 of 12). *Salmonella* was not detected in air or soil samples collected within the orchards (0 of 303). *E. coli* was isolated from 25 of 45 (56%) and 1 of 18 (5.6%) soil samples in the livestock and control orchards, respectively. *Escherichia coli* was isolated from 46 of 198 (23%) and 1 of 36 (2.8%) air samples in the livestock and control orchards, respectively. Members of the *Corynebacteriaceae* family, often associated with animals and the most prevalent bacterial family present in manure samples taken within the livestock operation, were, on average, more abundant (21%) in the phyllosphere of trees in the livestock orchard than in the control orchard (5.8%).

Significance: Livestock-associated microorganisms from animal operations may transfer into adjacent orchards; the food safety implication of this movement is unknown.

P3-62 Comparison of Commercial Sanitizer Monitoring Strips

TIAH GHOSTLAW, Amanda Chang, Paola Martinez-Ramos and Amanda Kinchla

University of Massachusetts-Amherst, Amherst, MA

Introduction: The FSMA Produce rule requires that all agricultural water remains free of pathogens, recommending sanitizer use to ensure this. There is currently limited guidance on the validation of appropriate tools that can help to monitor produce sanitizer for small scale farm operations.

Purpose: This study investigated different peracetic acid and hydrogen peroxide strips available on the market and evaluated which test strips are the most accurate against traditional titration methods.

Methods: This experiment evaluated the effect of organic load capacities (0, 100, and 500 COD) at different PAA concentrations (0, 30, 60, and 80 ppm). Titrations were performed with ceric sulfate and sodium thiosulfate to validate the amount of PAA and hydrogen peroxide in each sample. Following titration, test strips were tested according to the manufacturers' instructions and compared to the titration method for accuracy. Experiments were conducted in triplicate with three test strips per trial (n=3) and reported values were statically analyzed using a T-test on SAS program.

Results: Of the test strips used, three brands tested peroxide levels, and six tested PAA levels. With 0 COD, 33% of the peroxide strips and 25% of the PAA strips accurately reported the peroxide levels. With 100 COD, 25% of the peroxide strips and 17% of the PAA strips accurately reported the results. With 500 COD, 8% of the peroxide strips and 17% of the PAA strips accurately reported results.

Significance: This work is significant to the produce industry because it demonstrates that commercial PAA test strips are not able to accurately report sanitizer concentration levels and are negatively affected by the presence of organic load. Future work is needed to focus on alternative methods to support small growers in verifying sanitizer concentration in postharvest wash waters.

P3-63 Metagenomics Analysis of Antibiotic-resistance Genes Found on Radish Taproots Grown in Soils Amended with Antibiotic-treated Cattle

GISELLE KRISTI GURON, Monica Ponder and Amy Pruden

Virginia Tech, Blacksburg, VA

Introduction: Cattle are often treated with antibiotics, with their manure or composted manure used as a soil amendment for growing vegetables eaten raw. The effects of these agricultural practices on the produce microbial communities and antibiotic resistance genes (ARGs) are not well understood.

Purpose: Metagenomics of microbial communities of vegetables can provide overviews of dominant ARGs present on vegetables. The objective of this study was sequence metagenomes of the microbial communities from greenhouse radish bulb surfaces that were grown in soils amended with manure or compost originating from dairy or beef cattle of different histories of antibiotic administration.

Methods: Beef cattle were administered chlortetracycline, sulfamethazine, and tylosin while dairy cattle were administered cephapirin and pirlimycin. Manure was collected from antibiotic-treated cattle and nontreated control cattle. The four manure types were composted using a static method. The four manure types and four compost types were amended to two soil types (sandy loam and silty clay loam), in addition to fertilizer controls. Radish seeds were directly sown, and DNA was collected from microbes dislodged off the surfaces of mature taproots. After Illumina HiSeq, metagenomic sequences were mined for ARGs using the Comprehensive Antibiotic Resistance Database.

Results: Over 500 ARGs were identified among radish taproot surfaces. Some genes identified in all samples were *dfrE*, (trimethoprim resistance), *rosB* (polymyxin B resistance), and *rosA* (polymyxin B resistance). Genes that were not present on radishes grown in fertilizer control soils were *ramA* (multidrug resistance) and *marA* (induces transcription of efflux pump gene *acrAB*). However, those genes were present on radishes grown in soils amended with beef- and dairy-based manure and compost.

Significance: Further analysis will continue to identify ARGs. While ARGs will inevitably appear on vegetables regardless of biological soil amendment application, other ARGs can still be introduced into production by their use.

P3-64 Quantifying Sulfonamide-resistant Bacteria on Lettuce Grown in Soils Amended with Manure from Antibiotic-treated Cattle

GISELLE KRISTI GURON, Monica Ponder and Amy Pruden
Virginia Tech, Blacksburg, VA

Introduction: Administration of antibiotics to cattle presents a concern for the spread of antibiotic resistance, particularly when employing manure-based amendments to vegetable fields. In addition to direct selection of resistance to the antibiotics administered, effects on cross resistance of bacteria to other antibiotics should also be considered. Many antibiotic resistance genes (ARGs) are co-localized together on mobile genetic elements.

Purpose: Quantify *sul*1 ARGs and bacterial numbers on plates containing sulfamethoxazole associated with lettuce grown in soils amended with manure from cattle with different antibiotic administration histories.

Methods: Beef cattle were administered chlortetracycline, sulfamethazine, and tylosin while dairy cattle were administered cephapirin and pirlimycin. Manure from antibiotic-treated or control cattle were then mixed with soil before transplanting lettuce in the greenhouse (n=3). Fertilizer was applied to controls. At maturity, bacteria were removed from outer leaves and enumerated on R2A media and R2A containing sulfamethoxazole. Real-time PCR was used to quantify total microbes (16S rRNA genes) and *sul*1 ARGs in DNA from the same samples used for plating.

Results: Despite the difference in soil amendment, the R2A plate counts from lettuce surfaces were not significantly different (P>0.05, Wilcoxon). However, bacterial counts on R2A containing sulfamethoxazole were lower on lettuce grown in manure-amended soil compared to the fertilizer control (P< 0.043, Wilcoxon). There were no significant differences in *sul*1 numbers in relation to 16S rRNA numbers on lettuce surfaces among the manure types amended, regardless of antibiotic administration.

Significance: None of the manure treatments seemed to affect the *sul*1 numbers of the harvested lettuce leaves, regardless of the histories of antibiotic administration of the cattle from which the manure originated. This highlights that sulfonamide resistance may not only be selected for by sulfonamide administration, but other factors could be involved in the transfer of ARGs onto vegetables.

P3-65 Persistence of Non-O157 Shiga-toxigenic *Escherichia coli* on Fresh Produce Surfaces

Jennifer Green and **JITU PATEL**
U.S. Department of Agriculture, Beltsville, MD

Introduction: Illnesses attributed to nonO157 Shiga toxin-producing *Escherichia coli* (STEC) have increased in the past decade, with 22 foodborne outbreaks associated with nonO157 STEC. Lettuce and salad bars have been implicated in those outbreaks. Prevalence of the six major non-O157 STEC serotypes in agriculture soil and close association with fresh produce requires investigation on their persistence on fresh produce.

Purpose: This study evaluated the persistence of nonO157 STEC on fresh produce surfaces.

Methods: Twelve nonO157 serotypes (O26, O45, O103, O111, O121, O145) were evaluated for curli expression, hydrophobicity, and attachment to fresh produce (cabbage, romaine lettuce, spinach) leaves. Fresh produce leaves were cut to disc shaped pieces (22 mm diameter) and inoculated with 50 µl (10 micro-droplets of 5 µl each) of actively growing cultures. Inoculated fresh produce discs were incubated at 4, 10, and 22°C for 48 h and bacterial populations were analyzed at 0, 1, 4, 24, and 48 h by spiral plating on selective media. Curli expression and cell hydrophobicity was analyzed by Congo red (CR) dye and bacterial attachment to hydrocarbon (BATH) assay, respectively.

Results: Persistence of nonO157 serotypes varied with produce surface and incubation temperature. Recovery of attached STEC was two to three log CFU/g lower from their initial populations (~6.5 log CFU/g) when fresh produce was incubated for 24 to 48 h. Significant reductions in STEC populations were observed when spinach was incubated at 22°C for 48 h. *Escherichia coli* O26:H11 strain 5711 was recovered at significantly higher levels than *E. coli* O121:H19 strain 5705 on cabbage and romaine lettuce, following 48 h incubation at 4°C. In general, the curli-expressing ability of STEC strains did not influence bacterial attachment.

Significance: Persistence of nonO157 serotypes varied among STEC serotypes, produce surface, and incubation temperature. Bacterial attachment on produce surface was a complex phenomenon and knowledge regarding attachment will be helpful in the evaluation of contamination risk during processing and storage of fresh produce.

P3-66 Serotypes, Antimicrobial Resistance, and Genetic Correlations of *Escherichia coli* Isolated from Raw Kimchi Ingredients and Fermented Kimchi

YUKYUNG CHOI[1], Soomin Lee[1], Hyun Jung Kim[2] and Yohan Yoon[1]
[1]Sookmyung Women's University, Seoul, South Korea, [2]Korea Food Research Institute, Gyeonggi, South Korea

❖ ***Developing Scientist Competitor***

Introduction: Although kimchi is fermented food with lactic acid bacteria, foodborne outbreaks by consuming kimchi occurred occasionally and the major cause of the outbreaks was *Escherichia coli*.

Purpose: The objective of this study was to determine serotypes, antimicrobial resistance, and genetic correlation for *E. coli* isolated from raw kimchi ingredients and fermented kimchi.

Methods: One hundred raw kimchi ingredients [nappa cabbage (n=30), white radish (n=30), leek (n=20), and ginger (n=20)] and kimchi samples [nappa cabbage kimchi (n=20) and diced white radish kimchi (n=20)] were analyzed. Coliform and *E. coli* cell counts were enumerated on 3M™ Petrifilm™ at 37°C for 24 h. Typical *E. coli* colonies on the Petrifilm were identified by 16S rRNA analysis and multiplex PCR method. The identified *E. coli* was serotyped by immune assay. The antimicrobial susceptibilities of *E. coli* isolates were determined by a disc diffusion assay. To determine genetic correlation among the isolates, pulsed-field gel electrophoresis (PFGE) analysis were performed.

Results: *Escherichia coli* were isolated from nappa cabbage (1 of 30; 3.3%), nappa cabbage kimchi (3 of 20; 15%) and diced white radish kimchi (3 of 20; 15%). All isolates were then determined as *E. coli* by 16S rRNA analysis. The pathotypes were enterohemorrhagic *E. coli* (*stx* 2 positive) for *E. coli* from nappa cabbage and enteropathogenic *E. coli* (*eaeA* positive and *bfpA* positive or negative) for *E. coli* from all kimchi samples. Serotypes for the *E. coli* isolates were O6 for nappa cabbage and O20 for one colony from diced white radish kimchi. Isolated *E. coli* showed various antimicrobial susceptibility, and PFGE result showed close genetic correlation among the isolates.

Significance: This result suggests that characteristics of *E. coli* isolated from raw kimchi ingredients and fermented kimchi could be useful in understanding the cause of foodborne illness by kimchi consumption.

P3-67 Whole Genome Sequence Analysis of *Salmonella* Isolates from Alfalfa Seeds Implicated in Sprout Outbreaks

DEENA AWAD[1], Behzad Imanian[2], Peichen Liu[2], Yizhu Yan[2], Kristin M. Schill[1] and Tong-Jen Fu[1]

[1]U.S. Food and Drug Administration, Division of Food Processing Science and Technology, Bedford Park, IL, [2]Illinois Institute of Technology, Institute for Food Safety and Health, Bedford Park, IL

Introduction: Seeds are often linked to sprout outbreaks by epidemiological means, but confirmation through seed testing has not been successful in many cases. Studies are needed to determine whether testing sprouts grown from implicated seeds improves the detection of contamination.

Purpose: To determine whether testing sprouts permits improved detection of *Salmonella* in seeds implicated in sprout outbreaks, and evaluate the use of whole genome sequencing (WGS) in linking seed contamination to sprout outbreaks.

Methods: Alfalfa seeds obtained from two seedlots implicated in previous outbreaks (lot A, linked to 2009 *Salmonella* Oranienburg outbreak; and lot B, linked to 2016 *Salmonella* Muenchen/Kentucky outbreak) were analyzed for the presence of *Salmonella* following FDA BAM. Seeds from each lot were germinated in glass jars for two days at 30°C and germinated sprouts from each jar were analyzed in triplicate for *Salmonella*. Genomic DNA was isolated using the QIAGEN DNeasy kit. Paired-end sequencing libraries were prepared using Nextera XT and sequenced on an Illumina MiSeq. The sequences were analyzed using NCBI's Pathogen detection system and submitted to Genome Trakr.

Results: *Salmonella* was found in 1 of 10 seed and 1 of 8 sprout samples, or in 3 of 12 seed and 17 of 30 sprout samples from seedlot A or B, respectively. Based on the NCBI SNP tree results, *Salmonella* isolates IFSH00047-48 collected from sprouts grown from lot A clustered with *Salmonella* Oranienburg linked to the 2009 outbreak. IFSH00045-46 isolated from sprouts grown from lot B were found within a cluster that also contained a clinical *Salmonella* Muenchen strain 2016K-0108 collected by the CDC. IFSH00039-44 collected from seeds and sprouts from lot B clustered with clinical and sprout *Salmonella* Kentucky isolates linked to the 2016 outbreak.

Significance: Testing sprouts performed better than testing seeds in detecting *Salmonella* contamination. This is the first report of isolating *Salmonella* Muenchen in seeds implicated in the 2016 sprout outbreak.

P3-68 Microbiological Profile and Incidence of S*almonella* spp. on Cherry Tomato

MARLA LEAL-CERVANTES[1], Sofia Arvizu-Medrano[1], Montserrat Hernandez-Iturriaga[1] and Nanci E. Martínez-Gonzáles[2]

[1]Universidad Autónoma de Querétaro, Querétaro, Mexico, [2]Departamento de Farmacología, CUCEI, Universidad de Guadalajara, Guadalajara, Mexico

Introduction: Cherry tomato has become popular in the gourmet market due to its consumption as an ingredient in fresh salads. Unfortunately, a recall in 2012 related to the presence of *Salmonella* on cherry tomatoes and an outbreak in 2013 associated with the same pathogen in the United States of America, created an alert regarding the safety of the fruit. Information about the microbial risk at which people could be exposed by consuming cherry tomato is scarce.

Purpose: The objective of this study was to determine the microbiological quality including incidence of *Salmonella* spp. on cherry tomatoes at points of sale.

Methods: Samples of cherry tomatoes were collected from four supermarkets (157) and four local markets (83) from Querétaro, México. Fruit (50 g) were analyzed to quantify aerobic plate counts (APC), coliforms, molds/yeasts, and *Escherichia coli; Salmonella* presence was also investigated in 50 g samples. All microbiological analysis was done using Bacteriological Analytic Manual (BAM) methods. Nonparametric comparisons of microbial populations were carried out using the Wilcoxon-Kruskal-Wallis test.

Results: Populations of microbial indicator were higher in samples collected from local markets ($P<0.05$). Median values of APC, coliforms and molds/yeasts on tomatoes from supermarkets and local markets were 3.58 and 5.10, 1.02 and 1.52, 3.21 and 4.76 log CFU/g, respectively. *Escherichia coli* was present on samples from supermarkets (4.5%) and local markets (16.9%) at low levels (0.09 to 0.69 log MPN/g). *Salmonella* spp. was also detected in 16.6% (26 of 157) and in 8.4% (7 of 83) of the tested samples from supermarkets and local markets, respectively.

Significance: The presence of *Salmonella* was higher in tomatoes from supermarkets despite the fact that *E. coli*, an indicator of fecal contamination, did not reflect the same trend. This data set could be helpful to understand the risk for consumer's health associated with *Salmonella* and cherry tomatoes.

P3-69 Survival of *Salmonella enterica* on Mini Cucumbers

HUIHUI CHEN and Siyun Wang

University of British Columbia, Vancouver, BC, Canada

❖ ***Developing Scientist Competitor***

Introduction: Cucumbers have been identified as the cause for a recent salmonellosis. The ability of *Salmonella* to attach or internalize into produce may be a factor that make these produce items more likely to be sources of *Salmonella* contamination.

Purpose: The purpose of this study was to evaluate the survival capability of *Salmonella* on mini cucumbers.

Methods: Five strains of *Salmonella enterica* were inoculated onto mini cucumbers and subsequently incubated at 20°C for 8 days or at 4°C for 19 days respectively, to identify survival characteristics and capabilities. Three strains were isolated from irrigation water in Lower Mainland British Columbia, Canada (*Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Daytona). The other two were *Salmonella* Newport (FSL S5-639, a clinical isolate) and *Salmonella* Poona (S306). The survival of individual *Salmonella* strains was monitored using the one-step thin agar layer method.

Results: Overall, *S. enterica* was able to survive on cucumbers at both temperatures for an extended period of up to 8 or 19 days. Different *S. enterica* strains showed differential survival rates at both temperatures.

At 20°C, *Salmonella* Poona was identified to possess the strongest survival capability with the highest area under survival curve (AUC) of 50.74±0.431 days by log CFU, while the survival of *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Daytona, and *Salmonella* Newport all showed a slight decline. At 4°C *Salmonella* Enteritidis exhibited the strongest survival capability and there was an evident recovery of growth from the initial sharp decrease for all the other strains.

Significance: These results suggest that *Salmonella* can survive on cucumbers at room temperature and refrigeration. Unique survival characteristics among *Salmonella* strains at different temperatures increase our understanding of the factors influencing microbial safety of fresh produce and reveal that corresponding interventions need to be applied to eliminate contamination of produce with specific *Salmonella.*

P3-70 *Salmonella* Transfer Potential during Field-pack Handling of Cantaloupe

RACHEL PFUNTNER[1], Laura Truitt[1], Benjamin Chapman[2], Michelle D. Danyluk[3] and Laura Strawn[1]
[1]*Virginia Tech - Eastern Shore AREC, Painter, VA,* [2]*North Carolina State University, Raleigh, NC,* [3]*University of Florida, Lake Alfred, FL*

Introduction: Several cantaloupe-associated foodborne outbreaks have occurred due to cross-contamination. Minimal science-based metrics exist describing transfer coefficients for pathogen contamination onto melons during field-pack handling of cantaloupe.

Purpose: The purpose of this study was to quantify *Salmonella* transfer from contaminated gloves to cantaloupes, and vice versa, evaluating different contact times (5, 10, and 20 s) and pressures (none, mild, and vigorous).

Methods: Experiments were performed using store purchased cantaloupe with three types of gloves (nitrile, cotton, and rubber) typically worn by harvest crews. Two different transfer scenarios were evaluated: inoculated cantaloupe to clean glove and inoculated glove to clean cantaloupe. To simulate contamination in a dry environment, a dry inoculum protocol (inoculum mixed with sand) was used at a final concentration of 10^6 CFU/g. *Salmonella* populations from cantaloupes and gloves were enumerated on nonselective and selective agar supplemented with 50 µg/ml nalidixic acid. Transfer coefficients (TCs) were calculated, analyzed for analysis of variance, and significance was determined using least-squares significance test ($P \leq 0.05$) using SAS.

Results: *Salmonella* TCs were not significantly different between contact times for the two transfer scenarios. A positive association was observed between amount of contact pressure and *Salmonella* TCs. At vigorous pressure, rubber gloves transferred significantly more *Salmonella* to cantaloupes than nitrile and cotton gloves ($P \leq 0.05$). The rates of *Salmonella* transfer from inoculated cantaloupe to gloves were significantly higher than the rates of transfer from inoculated gloves to cantaloupe ($P \leq 0.05$). Similar to above, at vigorous pressure, cantaloupes transferred significantly more *Salmonella* to rubber gloves than nitrile and cotton gloves ($P \leq 0.05$).

Significance: The likelihood of *Salmonella* cross-contamination was influenced by different glove materials. Limiting the use of rubber gloves by harvest crews may minimize the risk of *Salmonella* transfer potential during field pack handling. The use of laundered cotton gloves or single-use nitrile gloves is recommended.

P3-71 Ability of the Top Seven Shiga-toxigenic *Escherichia coli* to Form Biofilms on Polystyrene and Stainless Steel Surfaces and to Survive within Dry Biofilms

Meining Cheng[1], Emelia Adator[1], Richard Holley[1], Tim McAllister[2] and **CLAUDIA NARVAEZ**[1]
[1]*University of Manitoba, Winnipeg, MB, Canada,* [2]*Agriculture and Agri-Food Canada, Lethbridge, AB, Canada*

Introduction: Biofilms are known to play an important role in bacterial survival and persistence in food processing environments.

Purpose: This work determined the ability of the top seven STEC strains to form biofilms on polystyrene and stainless steel; assessed their survival within dry biofilms; and quantified STEC transfer to lettuce pieces.

Methods: The ability of 14 STEC strains to form biofilms and survive on six well polystyrene (POL) plates and on two cm diameter 304-2B disk-shaped stainless steel (SS) coupons was examined. Biofilms on POL and SS were allowed to grow in low salt LB broth at 25°C for six days, washed three times with phosphate buffer, and dried for four hours. POL dry biofilms were stored for 2, 4, 6, and 30 days. SS dry biofilms were storage for 30 days. At each interval, dried lettuce pieces were placed over the dry SS and POL biofilms for two minutes. Lettuce pieces were collected and STEC enumerated. Thirty day old dry biofilms, negative for bacterial transfer, were enriched 24 hours to investigate bacterial survival. Scanning electron microscopy was used to evaluate dry biofilm formation on SS coupons. Experiments were conducted three times in duplicate.

Results: All STEC strains were able to transfer one to six log from POL dry biofilms to lettuce at two, four, and six days. Almost all serotypes showed decreased detachment with time. After 30 days on SS, all serotypes, but O26, detached and transfer to lettuce (0.3 to 3 log). After enrichment, all seven STEC serotypes were recovered from 30 day-old dry biofilms. Significant differences in transfer and survival were found among serotypes and with time ($P=0.001$).

Significance: Results showed that STEC can detach from dry biofilms and transfer to dried, fresh lettuce; and, after rehydration/enrichment, bacteria were able to fully recover. This suggested that dry biofilms could be an important factor in STEC cross-contamination in dry and wet food processing environments.

P3-72 Involvement of Surface Structures of Shiga Toxin-producing *Escherichia coli* O157:H7 and O104:H4 in Interactions with *Arabidopsis thaliana*

HYEIN JANG and Karl Matthews
Rutgers University, New Brunswick, NJ

Introduction: Bacterial cell surface structures, such as flagella, fimbriae, type 3 secretion system (T3SS), and exopolysaccharides, may have an impact on plant-pathogen interactions including attachment and colonization.

Purpose: This study aimed to investigate the influence of T3SS and surface polysaccharides of *Escherichia coli* O157:H7 and long polar fimbriae (Lpf) of *E. coli* O104:H4 on survival of the Shiga toxin-producing *E. coli* (STEC) on plant tissue.

Methods: Four week-old *Arabidopsis thaliana* (model plant) was dip-inoculated with STEC strains; *E. coli* O157:H7 EDL 933 wild-type and its isogenic T3SS-defective mutant, *E. coli* O157:H7 86-24 wild-type and its isogenic mutants (cellulose-deficient, colanic acid-deficient, and lipopolysaccharide (LPS)-truncated), and *E. coli* O104:H4 C3493 wild-type and Lpf-deficient mutants. Populations of each strain, expressed as a log CFU/g leaf tissue, on *Arabidopsis* plant were determined on day 0, 1, 3, and 5 postinoculation. Levels of plant defense response, based on expression of pathogenesis-related gene (*PR1*), was examined by reverse transcription quantitative PCR.

Results: The populations of wild-type *E. coli* O157:H7 and T3SS-defective mutant on *Arabidopsis* plants did not show a significant difference on day 1, 3, and 5 ($P>0.05$). The wild-type of *E. coli* O104:H4 and its corresponding Lpf mutants, similarly, showed no significant difference in the population at day 5 postinoculation ($P>0.05$). In regard to the influence of surface polysaccharides of *E. coli* O157:H7, however, wild-type strain survived better on *Arabidopsis* with two-fold lower expression of *PR1* gene compared with colanic acid-deficient and LPS-truncated mutants ($P<0.05$).

Significance: This study demonstrated that different surface polysaccharides of STEC can trigger the plant defense response, thereby affecting the survival of the human pathogens on plants. The overall results provided a better understanding about the behavior of STEC on plants, which is helpful for designing intervention strategies for providing safe crops.

P3-73 Migration and Growth of Enterohemorrhagic *Escherichia coli* from Inoculated and Accompanying Contaminated Vegetable Seeds to Sprouts or Seedlings

YUE CUI[1], Da Liu[1], Ronald R. Walcott[2] and Jinru Chen[1]
[1]*University of Georgia, Department of Food Science and Technology, Griffin, GA,* [2]*University of Georgia, Department of Plant Pathology, Athens, GA*

❖ ***Developing Scientist Competitor***

Introduction: Contaminated vegetable seed is a potential source of foodborne pathogens, such as enterohemorrhagic *Escherichia coli* (EHEC).

Purpose: This study was undertaken to determine whether EHEC on artificially-inoculated and accompanying-contaminated vegetable seeds could migrate to different tissues of sprouts/seedlings.

Methods: Alfalfa, fenugreek, tomato, and lettuce seeds were inoculated with EHEC F4546, K4499, H1730, or ATCC BAA-2326 by artificial-inoculation (IN) or accompanying-contamination (AC). For IN, overnight EHEC cultures were coincubated with vegetable seeds at 20°C for 5 h, whereas for AC, freeze-dried EHEC cells in sterile, sandy soil were coincubated with vegetable seeds at 20°C for one day. Contaminated vegetable seeds were subsequently germinated on 1% water agar at 25°C, in the dark. Populations of EHEC on different sprout/seedling tissues were determined every other day for five days.

Results: Approximately 91% (n=1,024) and 66% (n=1,024) of the sprout/seedling samples inoculated by IN and AC tested positive for EHEC, respectively. For IN, seed coats had the highest EHEC population (5.40 log), followed by radicle (5.18 log), cotyledon (4.80 log), and stem (4.41 log) tissues. For AC, however, EHEC populations associated with seed coat (3.63 log) and radical (3.48 log) tissues were similar, but significantly higher than those associated with cotyledon (3.16 log) and stem (2.94 log) tissues. IN fenugreek samples had the highest number of EHEC cells (5.79 log), followed by alfalfa (5.49 log), lettuce (5.26 log), and tomato (1.80 log) samples. For AC, a similar trend was observed, except that alfalfa samples had lower EHEC population (1.09 log) than lettuce samples. For both inoculation methods, the highest number of F4546 and lowest number of ATCC BAA-2326 cells were recovered from contaminated sprout/seedling tissues.

Significance: These data suggested that EHEC migrated from IN and AC vegetable seeds to various sprouts/seedlings tissues during germination. This indicated the importance of using pathogen-free seeds for vegetable sprout/seedling production.

P3-74 Formation of Disinfection Byproducts in Wash Water and Lettuce by Washing with Sodium Hypochlorite and Peracetic Acid Sanitizers

WAN-NING LEE and Ching-Hua Huang
Georgia Institute of Technology, Atlanta, GA

❖ ***Developing Scientist Competitor***

Introduction: Sodium hypochlorite (NaOCl) and peracetic acid (PAA) are being used for cleaning and sanitization in food processing, but their chemical behavior regarding disinfection byproducts (DBPs) formation during washing processes is still largely unknown. Even though PAA is known to have a lower tendency to form chlorinated DBPs than chlorine, the formation potential of DBPs still exists with PAA. Additionally, the side reactions that lead to DBP formation can consume disinfectants and lower their efficacy to inactivate pathogens, thereby increasing the chances of microbial contamination.

Purpose: This study was conducted to obtain a better understanding of the formation potential and distribution of DBPs in the wash water and fresh-cut lettuce after washing by PAA versus by NaOCl. This study also evaluated a wide variety of DBPs including conventional and emerging DBPs (a total of 46 target DBPs).

Methods: Procedures to simulate fresh-cut lettuce washing under produce processing conditions were conducted in the laboratory using three different concentrations of PAA (45 mg/liter, 85 mg/liter, and 100 mg/liter) and one dose of NaOCl (100 mg/liter). The wash water and washed fresh-cut lettuce were extracted and analyzed for the 46 target DBPs.

Results: The results demonstrated that washing by PAA generated fewer DBPs than washing by NaOCl in both wash water and fresh-cut lettuce. The majority of DBPs formed by PAA were aldehydes in the wash water, and aldehydes and trihalomethanes (THMs) in the washed lettuce. The majority of DBPs formed by NaOCl were haloacetic acids (HAAs), nitrogenous DBPs (N-DBPs) and aldehydes in the wash water, and THMs, HAAs, and aldehydes in the lettuce.

Significance: The comprehensive data will facilitate the development of safer produce washing processes and sanitizer applications and guide further research on DBPs in food.

P3-75 Comparison of Flume Wash and Single-pass Wash on the Formation of Disinfection Byproducts in Produce Processing

Ching-Hua Huang[1], **WAN-NING LEE**[1], Yaguang Luo[2] and Dave Morris[3]
[1]*Georgia Institute of Technology, Atlanta, GA,* [2]*U.S. Department of Agriculture–ARS, Beltsville, MD,* [3]*McEntire Produce, Columbus, SC*

Introduction: Washing fresh-cut produce with sanitizers, such as chlorine, is important to control microbial contamination, but may lead to formation of harmful disinfection byproducts (DBPs). The flume wash system is commonly used, but may encounter deteriorating water quality and high organic loads. The single-pass wash system represents a novel approach that may reduce the risk potential for pathogen cross-contamination.

Purpose: This study evaluated how the flume wash and single-pass wash systems differ in the potential to form DBPs during fresh-cut produce processing with chlorine sanitizer. The investigation included a range of conventional and emerging DBPs that are recognized for toxicity concerns to gain a comprehensive understanding.

Methods: The study was conducted at a produce processing plant that employed flume wash and single-pass wash systems in separate processing trains. Water and lettuce samples (n>28) were collected at multiple points from each train and analyzed for the levels of various DBPs (including trihalomethanes (THMs), haloacetic acid (HAAs), halonitriles, halonitromethanes, haloacetamides, chloral hydrate and chloropropanones) using appropriate analytical methods. Other monitored parameters included water pH, temperature, and chlorine level. The water supply to the plant and unwashed lettuce were analyzed as background samples.

Results: Results thus far show that the levels of DBPs were nearly 10 times lower in the single-pass water than in the flume water after washing shredded lettuce. The difference is less dramatic for DBP residues in lettuce, about 0.5 to 2 times lower for the single-pass wash system than for the flume. A final fresh water rinse at the end of the flume wash system was found to be important to reduce the final DBP residue levels in lettuce.

Significance: This study is among the first for a comprehensive evaluation of DBP levels from the flume wash versus single-pass wash systems. Results will be useful for the produce processing industry and research community working to improve food safety.

P3-76 Efficacy of Peroxyacetic Acid and Other Sanitizers for Ensuring Produce Safety

PRASHANT SINGH, Hang Qi and Yen-Con Hung
University of Georgia, Griffin, GA

Introduction: Incidences of foodborne outbreaks caused by consumption of contaminated fresh fruits and vegetables have increased in the recent past. *Escherichia coli* O157, *Listeria monocytogenes* and *Salmonella* spp. are pathogens frequently associated with produce related outbreaks. Contaminated fresh fruits and vegetables are matter of concern, as they are often consumed raw, thus posing as a threat to human health. Therefore, disinfection of produce with an effective sanitizer is one of the critical steps in ensuring produce safety.

Purpose: The objective of this study was to evaluate the efficacy of chlorine-based sanitizer (acidic electrolyzed water, near neutral electrolyzed water (NNEO) and bleach), lactic acid, VigorOx® 15 F&V (peracetic acid-based sanitizer), and DI water on produce inoculated with *E. coli* O157, *L. monocytogenes* and *Salmonella* Typhimurium DT104.

Methods: A five-strain cocktails of *E. coli* O157, *L. monocytogenes,* and *Salmonella* Typhimurium DT104 (each pathogen prepared separately) were used for surface inoculating produce (*E. coli* O157: romaine lettuce, lemons, tomatoes, and blueberries; *L. monocytogenes*: romaine lettuce and cantaloupe; *Salmonella* Typhimurium DT104: lemons, tomatoes, cantaloupe, and blueberries). Chlorine-based sanitizers at 100 ppm, VigorOx® 15 F&V at 45, 85, 100 ppm, lactic acid at 2% and DI water as control were used for washing inoculated produce with an automated produce washer for five minutes.

Results: All sanitizers showed a higher efficacy (2.8 to 6.8 log CFU) on produce samples with smoother texture (e.g., lemon, tomato, blueberry). Lower effectiveness (0.2 to 4.4 log CFU) was observed for produce with course surface (e.g., lettuce, cantaloupe). NNEO and VigorOx® 15 F&V at 100 ppm were most effective (2.2-log CFU/g reduction) on lettuce inoculated with *E. coli* O157. Whereas, VigorOx® 15 F&V at 100 ppm showed significantly (P<0.05) better results (4.4-log reductions) on cantaloupe inoculated with *L. monocytogenes*.

Significance: VigorOx® 15 F&V was found to be either superior or equally effective as chlorine-based sanitizers.

P3-77 Efficacy of Peracetic Acid for Inactivation of Foodborne Pathogens in Imazalil Fungicide Solutions Used in Citrus Packinghouses

Setareh G. Shiroodi, **ANNE-LAURE MOYNE** and Linda J. Harris
University of California-Davis, Davis, CA

Introduction: Green and blue molds can result in significant postharvest losses of citrus fruit. To reduce such losses, fungicides in solution are applied to fruit in recirculating tanks or sprays during packing. Compatible sanitizers may be used to maintain water quality and prevent cross contamination of fruits in packinghouses.

Purpose: Determine the minimum effective concentration of peracetic acid (PAA) to inactivate *Salmonella enterica* and *Listeria monocytogenes* in imazalil solutions.

Methods: Imazalil (300 ppm) was prepared with citrus packinghouse water and combined with 0, 10, 15, and 20 ppm PAA. *Salmonella enterica* or *L. monocytogenes* cocktails were separately inoculated (5.5 log CFU/ml) into imazalil preparations and held at 16 or 40°C for up to 5 minutes. At each time point, samples (n=6) were neutralized in Dey-Engley broth and plated onto appropriate agar; remaining samples were enriched at 37°C for 48 hours to confirm the presence of pathogens.

Results: At 16°C, ≥ five log reductions of *Salmonella* were achieved in ≥ five, three, or two minutes at 10, 15, or 20 ppm PAA, respectively in the packinghouse water (control). In the presence of imazalil, PAA efficacy was improved: ≥ five-log reductions were achieved in ≥ three minutes at 10 ppm, or ≥ two minutes at 15 or 20 ppm PAA. *Salmonella* could not be detected by enrichment after three minutes of exposure to ≥10 ppm PAA in the presence of imazalil. A five-log reduction of *L. monocytogenes* was not observed in the packinghouse water, even at 20 ppm PAA. However, in the presence of imazalil ≥ five-log reductions of *L. monocytogenes* were observed in ≥ three minutes at 15 or 20 ppm PAA; *L. monocytogenes* could not be detected by enrichment after exposure to 15 or 20 ppm PAA for five or three minutes, respectively. The efficacy of PAA was improved at 40°C; times to achieve ≥ five-log reduction in both pathogens was observed within one minute at 20 ppm PAA.

Significance: PAA has the potential to prevent cross contamination of fruit in imazalil applications used in citrus packinghouses.

P3-78 Injury and Recovery of *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* on Cantaloupe Rind Surfaces after Hydrogen Peroxide and Minimal Thermal Treatment

DIKE UKUKU[1], Sudarsan Mukhopadhyay[2], Modesto Olanya[3] and Brendan Niemira[4]
[1]U.S. Department of Agriculture-ARS-ERRC-FSIT, Wyndmoor, PA, [2]U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, [3]U.S. Department of Agriculture–ARS, Wyndmoor, PA, [4]U.S. Department of Agriculture-ARS, Wyndmoor, PA

Introduction: Produce surface structures vary and complicate decontamination treatments for reducing attached bacteria.

Purpose: This study investigated survival and recovery of injured *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* on cantaloupe rind surfaces after treatments with water at 22°C, water at 70°C, 3% hydrogen peroxide (H_2O_2) at 22°C, and a combination of 3% $H_2O_2$and water at 70°C for 300 s during storage at 5 and 22°C for seven days.

Methods: Whole melons were inoculated with *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes* inocula at 10^4 CFU/cm^2, respectively, and then treated for 300 s with water at 22°C, water at 70°C, 3% hydrogen peroxide (H_2O_2) at 22°C, or a combination of 3% H_2O_2 and water at 70°C. Surviving populations including injured bacteria were enumerated on different selective agar plates and the populations inactivated were calculated.

Results: Injured bacteria were among the surviving populations of *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes* recovered after treatments with 3% H_2O_2 alone; 20%, 15%, and 2%, respectively. Populations of injured pathogens on treated cantaloupe lasted for 24 h on rind surfaces stored at 22°C, but declined to <2 CFU/cm^2 on melons stored at 5°C. Percent of the surviving populations that were injured when melons were treated with 70°C water, averaged <2% for all pathogens tested. Hot water and a combination treatment with 3% H_2O_2 at 70°C led to a four log inactivation of all bacterial pathogens. Populations transferred to fresh-cut pieces were below detection, even after enrichment of the samples.

Significance: The results of this study indicated that surviving injured populations of *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes* on whole cantaloupes processed for 300 s at 70°C would not pose a microbial safety issue for fresh-cut pieces prepared from treated cantaloupes, when consumed immediately or after storage at 5°C for later consumption.

P3-79 Impact of Organic Practices on the Microbiological Quality and Safety of Lettuce in São Paulo, Brazil

DANIELE FERNANDA MAFFEI, Debora Andrade Moreira, Mariza Landgraf and Bernadette DGM Franco
University of São Paulo, São Paulo, Brazil

Introduction: The consumption of organic products has increased worldwide in consequence of the demand for healthy food, without chemical residues. However, organic farming practices, such as the use of animal manure, can increase the risk of contamination by pathogenic microorganisms. Little is known about the microbiological characteristics of organic vegetables produced and retailed in the city of Sao Paulo, Brazil.

Purpose: As part of a large project on microbiological risks associated with organic vegetables produced and retailed in Sao Paulo, Brazil, the aim of this work was to report results on the quality and safety of organic lettuce samples (*Lactuca sativa* L.) collected from three certified farms located in the vicinity of the Sao Paulo, where chicken manure was regularly used as fertilizer.

Methods: Thirty samples of different lettuce varieties (looseleaf, red looseleaf, butterhead, and romaine lettuce), collected at three organic farms, were submitted for enumeration of total coliforms and *Escherichia coli* using the standard MPN method. Samples were also tested for *Salmonella* spp. using ISO 6579:2002 method.

Results: All samples were positive for total coliforms (average 2.6±0.7 log MPN/g) and *E.coli* was detected in 14 (47%) samples (average 1.0±0.6 log MPN/g). None of the samples was positive for *Salmonella* spp. in 25 g.

Significance: Results indicated that the samples presented good microbiological quality and were in accordance with the current limits established by the Brazilian Surveillance Agency for fresh vegetables (<2 log MPN/g for thermotolerant coliforms and absence of *Salmonella* spp./25 g). These preliminary results suggested that chicken manure used as fertilizer had little, if any, impact on the safety of organic lettuce produced on the tested farms.

P3-80 Survival and Growth of Foodborne Pathogens in Fruit Juice

Lindsey DeFrain, **HAMOUD ALNUGHAYMISHI** and Elliot Ryser
Michigan State University, East Lansing, MI

✦ ***Undergraduate Student Award Competitor***

Introduction: Given the trend toward more healthy lifestyles, consumption of freshly made juice is increasing which brings new food safety concerns.

Purpose: This study aimed to evaluate growth and survival of *Listeria monocytogenes, Salmonella*, and *Escherichia coli* O157:H7 in freshly made orange, apple, and pear juices.

Methods: Juices from locally obtained oranges, apples, and pears were extracted using a Hamilton Beach 67608 Juicer after which sugar content and pH were measured using a refractometer and pH meter, respectively. The juices were inoculated with three-strain virulent cocktails of *L. monocytogenes, Salmonella,* and *E. coli* from recent outbreaks to contain ~ three log CFU/ml. Samples of each juice were then stored at 4°C, 10°C, and 22°C with subsamples collected daily over a period of five days for microbiological analysis. *Listeria monocytogenes, Salmonella,* and *E. coli* O157:H7 were enumerated by surface-plating appropriate dilutions on trypticase soy agar + yeast extract (TSAYE) containing 0.1% (w/v) esculin and 0.05% (w/v) ferric ammonium citrate, TSAYE containing 0.05% ferric ammonium citrate and 0.03% sodium thiosulfate, and Sorbitol MacConkey Agar after 24 h of incubation. All experiments were conducted in triplicate.

Results: None of the pathogens grew in any of the juices with significantly greater declines seen for *Listeria* (0.5 to 1.7 log) compared to *Salmonella* (0.1 to 0.7 log) and *E. coli* O157:H7 (0.1 to 0.4 log). By day five, both orange and apple juice had visible mold at 22°C. The pH of the apple, orange and pear juice was 3.80±0.1, 3.63±0.2 and 3.95±01, respectively, while the sugar content was 12, 12, and 14°Brix, respectively.

Significance: This study demonstrates the ability of foodborne pathogens to survive in apple, orange, and pear juices during storage at different temperature. Therefore, measures to prevent or eliminate food pathogens in fresh juices and their preparation areas are needed to better ensure end product safety.

P3-81 Evaluation of Four Assessment Methods Used to Identify Foodborne Pathogens Prior to the Harvest of Fresh Produce

PAULA RIVADENEIRA[1], Martha Ruedas[1], Teresa Reyes[1], Elene Stefanakos[1] and Robert Buchanan[2]
[1]University of Arizona, Yuma, AZ, [2]University of Maryland, Department of Nutrition and Food Science and Center for Food Safety and Security Systems, College Park, MD

Introduction: Within seven days before harvest, produce growers conduct foodborne pathogen testing of their crops to ensure food safety. Currently, there is no standardized pattern to collect these samples.

Purpose: We tested the efficacy of four sampling patterns in produce fields in southern Arizona to determine which is best at identifying foodborne pathogens.

Methods: [1.] Using random, stratified random, Z-pattern, and targeted preharvest assessment techniques, we collected 747 leafy green samples from 10 fresh produce fields 24 to 72 h after they tested positive for Shiga toxin-producing *E. coli* (STEC) or *Salmonella*. [2.] We conducted a controlled blind trial by contaminating a romaine lettuce field with dairy cow manure tea inoculated with *E. coli* at 10^6CFU/ml. After 48 h, we collected blind samples using random, stratified random, and Z-pattern techniques. [3.] We conducted another controlled blind trial and collected the samples using the same three sampling patterns, but after only 12 h of overnight exposure.

Results: [1.] One field tested positive for *Salmonella* (one sample), *E. coli* O157 (one sample), and generic *E. coli* (four samples). Two other fields tested positive for generic *E. coli* (two samples each). The time lapse between the initial positive test results and our resampling of the field may have allowed environmental factors to eliminate pathogens. [2.] We did not recover *E. coli* during the first blind trial. [3.] We recovered two positive samples using the random sampling method, three using stratified random, and two using Z pattern. None of the sampling types performed better than the others, but the results indicated that ultraviolet radiation may have destroyed pathogens during our first two studies.

Significance: Our data indicate that preharvest sampling patterns do not differ in efficacy, and that environmental factors may play an important role in pathogen persistence, particularly in the southwest desert region.

P3-82 Prevalence of Foodborne Pathogens on Small Mixed Crop-Livestock Farms in Arizona

PAULA RIVADENEIRA[1], Martha Ruedas[1], Teresa Reyes[1], Elene Stefanakos[1] and Robert Buchanan[2]

[1]University of Arizona, Yuma, AZ, [2]University of Maryland, Department of Nutrition and Food Science and Center for Food Safety and Security Systems, College Park, MD

Introduction: Small mixed crop-livestock farms pose unique food safety risks as a result of the close proximity of livestock to produce.

Purpose: The purpose of this study was to document the prevalence of *Salmonella*, *Escherichia coli* O157, and nonO157 Shiga toxin-producing *E. coli* in small mixed crop-livestock farms in Arizona that sell their products, including milk, meat, eggs, and/or produce, at farmers' markets and other local venues.

Methods: We collected 510 samples from 16 farms during each growing season from spring 2015 to summer 2016. We aseptically collected water, soil, leafy greens, tomatoes, domestic and livestock animal feces, and various types of compost, and tested them using traditional culture and end point PCR techniques.

Results: One cow fecal sample tested positive for *E. coli* O157, but all other samples tested negative for all the pathogens we attempted to detect. We believe that the lack of positive samples may be explained by the stringent food safety protocols that were implemented at each farm. Our results were similar to those found by other researchers conducting the same study in other regions of the United States.

Significance: We conducted the first pathogen survey in the southwest growing region of small biodiverse farms that integrate produce and livestock production, particularly those involved in the direct farm to fork pathway. The data suggest that the food safety protocols being implemented by these farms are quite effective.

P3-83 Rotational Grazing of Sheep within Organic Crop Fields: What is an Ideal Waiting Period to Minimize Food Safety Risks?

LAURA PATTERSON[1], Nora Navarro-Gonzalez[2], Peiman Aminabadi[2], Elizabeth Antaki[3], Michele Jay-Russell[2] and Alda Pires[1]

[1]University of California-Davis, Department of Population Health & Reproduction, Davis, CA, [2]University of California-Davis, Western Center for Food Safety, Davis, CA, [3]Western Center for Food Safety, University of California-Davis, Davis, CA

Introduction: Integrated crop-livestock farms raise livestock and crops and use animals to graze cover crops. These operations are considered sustainable because grazing enhances soil fertility. However, manure deposited by livestock may introduce foodborne pathogens into the soil.

Purpose: The objectives of this study focused on assessing the die-off of generic *Escherichia coli* in the soil, after fields were grazed by sheep; determining the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) in the sheep; and assessing the genetic relatedness between *E. coli* isolates from soil and sheep feces.

Methods: Sheep grazed cover crops at an organic farm prior to planting Field A (onions) and Field B (melons). Fecal samples (*n*=24) were collected from sheep on day 0 and 56. Soil samples (*n*=192) were collected weekly for the first five weeks, then monthly for eight visits total. *Escherichia coli* strains were compared by pulsed-field gel electrophoresis (PFGE).

Results: Results showed a 3.0-log reduction of mean generic *E. coli* concentration (Most Probable Number, MPN) in the soil from the peak of 3.7 log MPN/g at 48 days post sheep (DPS) grazing to 0.67 log MPN/g by 111 DPS in Field A. Field B showed a 2.6-log reduction of mean generic *E. coli* concentration from the peak of 3.5 log MPN/g at 14 DPS to 0.95 log MPN/g by 84 DPS. STEC prevalence in the sheep flock was 4.2% (1 of 24). Closely related *E. coli* strains were found in soil and feces.

Significance: Developing research-based waiting periods between grazing and harvest is important to inform best practices for farmers and food safety stakeholders. Although conditions vary by season and region, this study indicates that the mean generic *E. coli* MPN/g levels in the soil after grazing are below 1.0 log MPN/g by the 120 day standard used by the USDA National Organic Program for raw manure application.

P3-84 Isolation of *Salmonella* and Detection of Generic *Escherichia coli* populations from South Florida Surface Waters

TRAVIS CHAPIN and Michelle D. Danyluk

University of Florida, Lake Alfred, FL

Introduction: The microbial quality of agricultural water may be influenced by various factors. *Salmonella*-contaminated surface waters may lead to preharvest contamination if water contacts the harvestable portion of the crop.

Purpose: The purpose of this study was evaluate the microbial quality of South Florida surface waters.

Methods: Water samples (1 liter) were collected monthly for twelve months at eight study sites along canals in South Florida. Samples were analyzed for turbidity, air temperature, water temperature, pH, and oxidation-reduction potential. Precipitation and relative humidity data were collected from the Florida Automated Weather Network. Samples were enumerated for total aerobic plate count, total coliforms and generic *Escherichia coli*. Samples were enumerated for *Salmonella* with a three-by-three MPN. Samples were inoculated in tetrathionate broth and Rappaport-Vassiliadis broth dilutions and incubated at 35 or 42°C for 24 or 48h, respectively, prior to streaking onto XLT-4 and Chromagar *Salmonella*. Presumptive *Salmonella* colonies were confirmed by *invA* PCR and serotyped.

Results: *Salmonella* was isolated from 26% of samples (25 of 96). *Salmonella* concentrations ranged from 0.50 to 3.00 log MPN/100 ml. Coliform populations ranged from 2.60 to 5.20 log MPN/100 ml. Aerobic plate counts were 3.80 to 6.10 log CFU/100 ml. *Salmonella* detection was not associated with *E. coli* or coliform populations or chemical or physical water characteristics. Geometric means of *E. coli* ranged from 0.88 to 1.82 log MPN *E. coli*/100 ml and statistical threshold values ranged from 1.59 to 2.47 log MPN *E. coli*/100 ml.

Significance: *Salmonella* populations are present in South Florida surface waters and cannot be predicted by total coliforms, generic *E. coli* or chemical and physical water characteristics. These data suggest these South Florida surface water sources will meet Produce Safety Rule water quality criteria; additional work is needed to evaluate at least 20 samples per source and use methods required by the PSR.

P3-85 Application of Cinnamon Oil Nanoemulsion to the Control of Foodborne Bacteria Such as *Listeria spp.* and *Salmonella spp.* on Honeydew Melon

SUMIT PAUDEL, Kanika Bhargava and Hari Kotturi
University of Central Oklahoma, Edmond, OK

❖ ***Developing Scientist Competitor***

Introduction: *Listeria* and *Salmonella* related recalls and outbreaks are of major concern to melon industry. Cinnamon oil have shown their usefulness in food treatment due to strong antifungal, antiviral, and antibacterial activities. However, its applications are limited due to poor solubility of cinnamon oil in water. Utilization of cinnamon oil nanoemulsion may offer effective antimicrobial washing treatment to melon industry.

Purpose: The purpose of this study was to test the antimicrobial efficacy of cinnamon oil nanoemulsion on melons against major foodborne pathogens such as *Listeria monocytogenes* and *Salmonella enterica*.

Methods: Different formulations of cinnamon oil nanoemulsion was made by ultrasonication using Tween 80 as emulsifier. Nanoemulsion exhibiting the smallest oil droplets was applied. Oil droplets were characterized for particle size by dynamic light scattering. Microbroth dilution assay was performed on three strains each of *L. monocytogenes* and *S. enterica* to find out the antimicrobial efficacy of cinnamon oil nanoemulsion. Honeydew melons were artificially inoculated with the above mentioned strains followed by treatment in nanoemulsions (control, 0.1%, and 0.25%) for one minute. Samples were dried and enumerated after one hour of treatment on selective media (PALCAM and XLD agar).

Results: The average diameter of nanoemulsions was 19.6 nm. Minimum inhibitory concentration (MIC) of cinnamon oil nanoemulsion for both *Listeria* and *Salmonella* strains was 0.078% v/v and 0.039% v/v, respectively. The minimum bactericidal concentration was 0.078% v/v for both. Compared to the water control, melon showed one-log reduction in *L. monocytogenes* and two-log reduction in *S. enterica*, respectively. 0.5% v/v of nanoemulsions might yield better log reduction in future trials.

Significance: The data suggest that cinnamon oil nanoemulsion can be used as an effective natural microbial control agent for honeydew melons.

P3-86 Differential Growth of *Listeria monocytogenes* and *Salmonella enterica* Serovar Typhimurium in Sterile Juice from Fresh-cut Produce

SAMANTHA BOLTEN[1], Ganyu Gu[2] and Xiangwu Nou[1]
[1]U.S. Department of Agriculture–ARS, Beltsville, MD, [2]Virginia Tech, Painter, VA

Introduction: Ability of bacterial growth on fresh-cut produce under temperature abuse conditions is a food safety concern. Exudate material from fresh cut produce is often implicated as a supplementing factor for bacterial growth, particularly the growth of pathogens associated with foodborne illness. This study focused on evaluating the growth of foodborne pathogens in the juice of several produce commodities.

Purpose: The purpose of this study was to evaluate the potential for growth by *Listeria monocytogenes* and *Salmonella enterica* in the sterile juice extracted from several fresh cut produce commodities.

Methods: Juice was extracted from various fresh-cut produce commodities (including cabbage, pineapple, green pepper, cucumber, tomato, radish, carrot, broccoli, onion, romaine lettuce, celery and cantaloupe) and sterilized by filtration through 0.22 µm filters. Overnight cultures of *S. enterica* and *L. monocytogenes* were starved, inoculated into produce juices, and incubated for 24 hours at 37°C in a microplate reader. Bacterial growth (absorbance at OD 600) was measured over a period of 24 hours. Samples were also plated after 24 hours of incubation to estimate the bacterial population, with a detection limit of 1.7 log CFU/ml. The total proliferation was determined by comparing the population at the end of growth to that of the inoculum.

Results: Results found the overall population growth of *S. enterica* in the produce commodities tested was significantly higher than the growth of *L. monocytogenes* ($P<0.01$). Differential results show that *S. enterica* grew better in juice extracted from tomato than *L. monocytogenes*. Neither bacteria showed significant growth in the juice of cabbage, onion, broccoli, and pineapple.

Significance: This information can be used to evaluate the potential for growth of bacterial pathogens on fresh cut produce during processing and storage.

P3-87 Forced Air-Ozone Reactor Combined with Sequential Advanced Oxidative Process to Inactivate *Listeria monocytogenes* on Apples Destined for Candy Apple Production

KAYLA MURRAY, Fan Wu and Keith Warriner
University of Guelph, Guelph, ON, Canada

❖ ***Developing Scientist Competitor***

Introduction: In 2014 there was an outbreak of listeriosis associated with contaminated candy apples, thereby requiring a preventative control intervention to be applied. Simple washing apples in sanitizing solutions is limited and in any event not preferred given that fruit requires to be dried to ensure adhesion of the candy layer. Therefore, alternative, aqueous-free, decontamination method(s) are required.

Purpose: This study was conducted to develop an ozone based treatment followed by advanced oxidative process (AOP) to inactivate *L. monocytogenes* introduced onto and into apples destined for candy apple production and to determine the fate of *L. monocytogenes* during posttreatment storage.

Methods: A forced air ozone reactor was constructed that introduced antimicrobial gas through an airstream into a column of apples that had been surface and subsurface inoculated with *L. monocytogenes*. The AOP reactor combined hydrogen peroxide mist in a chamber illuminated with ultraviolet light at 254 nm and ozone generation at 174 nm. The optimized treatments were applied to decontaminate apples that were used to prepare candied apples. The fate of *L. monocytogenes* on the candied apples during storage at 4 or 22°C was assessed.

Results: The ozone treatment optimized with respect to air flow and treatment time resulted in 1.90 to 2.40 log CFU reduction of target bacteria throughout the apple bed, but negligible decrease in subsurface populations. AOP, using 6% hydrogen peroxide vapor introduced at 48°C, and ultraviolet illumination for 30 s supported a four log CFU reduction of *Listeria* on the surface and 0.86 log CFU of internal populations. When combined in a sequential process, residual levels did not undergo outgrowth in the produced candy apples stored at 4 or 22°C.

Significance: A sequential process based on ozone and AOP provided an effective prevention control intervention for apples and could be extended to other fresh produce.

P3-88 Reduction of *Escherichia coli* O157:H7, *Listeria monocytogenes*, or *Salmonella* spp. on Whole Yellow Onions Exposed to Hot Water or Diced Onions Exposed to Lime Juice

Vanessa Lieberman[1], **ETHAN W. MORGAN**[2] and Linda J. Harris[2]
[1]*University of California-Davis, Food Science and Technology, Davis, CA,* [2]*University of California-Davis, Davis, CA*

Introduction: In-home or food service antimicrobial treatment options for fresh produce are limited. For some types of produce, controlled exposure to hot water or organic acids may provide a means to reduce food safety risks in these settings.

Purpose: This study was performed to identify hot water or lime juice treatments for whole (unpeeled) or diced onions, respectively, that significantly reduced levels of inoculated pathogens.

Methods: Separate cocktails of rifampin-resistant *Escherichia coli* O157:H7, *Listeria monocytogenes,* or *Salmonella* spp. were prepared in sterile water. The stem, equator, or root end of the outer papery skin of whole yellow onions (*Allium cepa*) was inoculated at six log CFU/onion. After drying for 30 minutes and storage for zero to six days, onions were immersed in water at 100°C for five seconds or at 85°C for 5 to 180 seconds. Freshly diced yellow onions (20 g) were inoculated at three log CFU/g, 15 ml of commercially-prepared lime juice were added, and the mixture held at ambient temperatures for zero to 30 minutes. Samples (n=6) were plated onto tryptic soy agar and CHROMagars (O157, *Listeria*, and *Salmonella*), all supplemented with 75 µg/ml rifampin.

Results: Exposure of whole onions at 100°C for five seconds reduced populations of *Salmonella* by > five log CFU/onion at the stem and equator, but not the root end. Reductions of > five log CFU/onion of *E. coli* O157:H7, *L. monocytogenes* or *Salmonella* spp. at the root end were achieved when onions were exposed to 85°C hot water for 60 seconds, but not consistently at 45 seconds. After 30 minutes, less than one log CFU/g (0.41, 0.33, or 0.82 log CFU/g) declines of *E. coli* O157:H7, *L. monocytogenes,* or *Salmonella* spp., respectively, were observed in diced onions mixed with lime juice.

Significance: Short exposure to hot water can significantly reduce pathogens on the surface of whole onions; but, soaking diced onions in lime juice had little effect.

P3-89 Cold Plasma Inactivation of *Escherichia coli* and *Salmonella* spp. on Golden Delicious Apples

SIQIN LIU, Agnes Kilonzo-Nthenge, Ankit Patras and Yannam Sudhear
Tennessee State University, Nashville, TN

Introduction: The rising demand for fresh produce poses the challenge for the food industry in supplying safe produce with minimal processing. Cold atmosphere plasma (CAP) is an emerging nonthermal technology applied to control foodborne pathogens.

Purpose: The aim of this study was to investigate the effect of CAP on *Escherichia coli* and *Salmonella* spot inoculated on the surface of golden delicious apples.

Methods: Filtered air at a pressure of four bars and at a flow-rate of 17 liters/min were used for the plasma generation. The process parameters considered were plasma exposure time for 0.5, 1, 2, 3, and 4 minutes at a fixed distance of 35 mm and fixed input power of 200 Watts. Each treated spot inoculation was swabbed with wet sterile cotton swabs and a series of dilutions were performed. Each dilute (0.1 ml) was spread in duplicates on TSA plates and incubated at 37°C for 24 hours. All plate count data was converted to log CFU/ml.

Results: All treatments resulted in significant (P<0.05) time-dependent reduction compared to untreated control. The reductions ranged from 1.4 to 5.3 log CFU/ml and 0.6 to 5.5 log CFU/ml for *E. coli* (ATCC 25922) and *E. coli* (ATCC 11775), respectively. The reduction of *Salmonella* Typhimurium (ATCC 13311) and *Salmonella* Choleraesuis (ATCC 10708) ranged from 2.8 to 4.8 and 1.3 to 5.3 log CFU/ml, respectively. *Escherichia coli* and *Salmonella* were inactivated by approximately 5.5 log CFU/ml at four minutes at a distance of 35 mm. The microbial inactivation effect of cold plasma treatment can be attributed to several synergistic mechanisms, including the generation of ultraviolet irradiation, ozone, charged particles, and oxygen radicals, in addition to other reactive species.

Significance: According to our results, CAP treatment was effective in the inactivation of *E. coli* and *Salmonella* on apples. However, further investigation is needed for elucidating the organoleptic and nutritional quality changes of fresh produce after plasma treatment.

P3-90 Influence of Chemical Pretreatment on Wooden Sticks Alone and in Caramel Apples on *Listeria monocytogenes* Survival

CHRISTINA K. CARSTENS[1], Joelle K. Salazar[2], Vriddi M. Bathija[3], Sartaj S. Narula[3], Peien Wang[3] and Mary Lou Tortorello[2]
[1]*U.S. Food and Drug Administration, Bedford Park, IL,* [2]*U. S. Food and Drug Administration, Bedford Park, IL,* [3]*IIT Institute for Food Safety and Health (IFSH), Bedford Park, IL*

❖ ***Developing Scientist Competitor***

Introduction: The 2014 to 2015 listeriosis outbreak involving caramel apples has led to various studies intended to establish and reduce listeriosis risk associated with consumption of caramel apples. *Listeria monocytogenes* can both survive and grow in inoculated caramel apples. Additional research is necessary to determine methods for the control of this pathogen in caramel apples.

Purpose: This study determined the effectiveness of various chemical pretreatments for the reduction of *L. monocytogenes* on wooden sticks and in caramel apples.

Methods: Wooden sticks were soaked, overnight, in solutions of 1, 3, 5, 10, and 20% (w/v) sodium benzoate, Nisaplin, and ascorbic acid and dried for two hours. The sticks were then inoculated with seven log CFU/stick of a *L. monocytogenes* cocktail composed of three strains isolated from the caramel apples outbreak and dried for another two hours. Sticks were either stored at 25°C for eight days or used in caramel apple production. At timepoints, sticks or apples were homogenized with BLEB for *L. monocytogenes* recovery by enrichment and enumeration on Brilliance *Listeria* Agar (Oxoid) plates. Experiments were conducted twice with triplicate samples for each condition.

Results: The *L. monocytogenes* populations on wooden sticks that received 3% ascorbic acid and 5% sodium benzoate treatments had significant decreases in population compared to controls after two hours drying (4.92±0.21 and 4.27±0.23 log CFU/stick, P<0.05, respectively). After 24 h at 25°C, the *L. monocytogenes* populations exposed to the 1, 3, and 5% ascorbic acid treatments had decreased below the level of enumeration (two log CFU/stick) and was absent in enrichments. In caramel apples, 10 and 20% ascorbic acid decreased the populations of *L. monocytogenes* up to 48 hours; however subsequent timepoints revealed no significant differences compared to the controls.

Significance: This study will help to ascertain a functional stick pretreatment that may reduce *L. monocytogenes* growth on caramel apples.

P3-91 Disinfection of Alfalfa Sprouting Seed Using a Treatment Compliant with Organic Production Requirements

PASCAL DELAQUIS[1], Susan Bach[1], Steve Orban[1], Carmen Wakeling[2] and Siyun Wang[3]
[1]Agriculture and Agri-Food Canada, Summerland, BC, Canada, [2]Eatmore Sprouts & Greens Ltd., Courtenay, BC, Canada, [3]University of British Columbia, Vancouver, BC, Canada

Introduction: Sprouting seed disinfection treatments recommended by Canadian regulatory agencies require the application of concentrated chlorine (2,000 ppm) or hydrogen peroxide (8%) solutions. Occupational safety and health risks, environmental concerns, and increasing demand for organically certified products mandate the development of alternative disinfection strategies.

Purpose: The purpose of this study was to evaluate the antimicrobial efficacy of an alfalfa seed disinfection treatment based on the application of mild heat and chemicals compatible with organic production.

Methods: Alfalfa seed, inoculated with five log cfu/g *Salmonella enterica, E. coli* O157:H7, and *Listeria monocytogenes*, was treated with: 1) 2,000 ppm chlorine for 20 min; 2) 8% H_2O_2 for 10 min; and 3) 10 min in water at 50°C followed by 10 minutes in 2.0% H_2O_2+0.1% acetic acid. Surviving populations were estimated with selective media and by nonselective enrichment procedures. Seed germination rates and yields were also measured.

Results: Treatment with 2,000 ppm chlorine for 20 min reduced populations of *S. enterica* (3.19±0.13 log cfu/g), *E. coli* O157:H7 (3.20±0.07 log cfu/g) and *L. monocytogenes* (3.83±0.14 log CFU/g). In contrast, treatment with 8% H_2O_2 for 10 min or a 10 min soak at 50°C followed by 10 minutes in 2.0% H_2O_2+0.1% acetic acid consistently reduced populations by five log cfu/g, but all species were recovered by enrichment. Germination rates and yields were not significantly (P>0.05) affected by the treatments.

Significance: Populations of *S. enterica*, *E. coli* O157:H7 and *L. monocytogenes* on alfalfa seed were reduced by five log cfu/g using a treatment compliant with organic food production principles. In parallel with other seed disinfection strategies that employ aqueous sanitizers, the treatment did not ensure complete pathogen eradication.

P3-92 Practices and Conditions which Promote Persistence of *Listeria monocytogenes* on Equipment Surfaces and Transfer to Cantaloupes in the Packing Environment

ESMOND NYARKO[1], Kalmia Kniel[1], Bin Zhou[2], Cheryl East[2], Eric Handy[2], Yaguang Luo[2], Patricia Millner[2] and Manan Sharma[2]
[1]University of Delaware, Newark, DE, [2]U.S. Department of Agriculture–ARS, Beltsville, MD

Introduction: Investigation of the 2011 United States listeriosis outbreak associated with contaminated cantaloupes revealed that transfer of *Listeria monocytogenes* (*Lm*) from equipment surfaces to melons in the packing facility was a potential route of contamination.

Purpose: This study examined *Lm* persistence on unclean materials and different types of surfaces and the transfer to cantaloupes in a simulated packing environment.

Methods: Clean and unclean (0.5 ml cantaloupe extract; dried 6 h) surfaces of nylon brush bristles (1 g), and 16 cm^2 of conveyor belts (polyvinyl-chloride, polyurethane, and nitrile-rubber), and foam-pads (16 cm^2) were prepared. Each surface was inoculated with a multistrain *Lm* cocktail (4.5 log CFU/ml), and subsequently stored at 25°C. *Lm* populations on surfaces were recovered for up to 15 days. A separate set of inoculated unclean surfaces received water (1 ml) 24 h prior to each enumeration event. Conveyor belt and foam pad materials were spot-inoculated (50 µl; 2.5 log CFU/surface) and 15 consecutive wet melons were manually rolled over each spot. Melons were tested for presence/absence of *Lm*.

Results: *Lm* populations decreased from 4.5 on day zero to 0, 2.3±0.5, and 3.1±0.2 log CFU/surface on clean conveyor belts, brush, and foam-pad materials, respectively, by day 10. *Lm* was still present on clean surfaces after 21 days. However, *Lm* populations remained unchanged (4.5 log CFU/surface) on unclean surfaces from days zero to 14. Multivariate ANOVA revealed that unclean surfaces significantly (P<0.05) promoted persistence of *Lm* compared to clean surfaces. Comparison of clean surfaces revealed that foam pad and brush significantly (P<0.05) promoted *Lm* persistence more than polyurethane, polyvinyl-chloride, and nitrile-rubber. An ANOVA of probability distributions of contaminated melons (n=90 melons per surface) revealed that foam pad contaminated significantly (P<0.05) more melons (78±5%) than polyvinyl-chloride (55±11%), which was approximately equivalent to polyurethane (47±9%) and nitrile-rubber (33±10%) belts. There was no correlation between the presence of *Lm* on melons and the order in which they were exposed to contaminated surfaces.

Significance: Unclean surfaces and materials that retain water promoted persistence and widespread contamination of melons.

P3-93 Evaluation of Microbiome Present in Bagged Spring Mix Salad

CHAO LIAO and Luxin Wang
Auburn University, Auburn, AL

❖ ***Developing Scientist Competitor***

Introduction: Outbreaks and recalls of fresh salad products have increased in the past decade. The different natural microbiota harbored in salad products may affect shelf-life and have an antagonistic capacity against foodborne pathogens contaminated during the process of food production.

Purpose: The aim of this research is to investigate the diversity and abundance of the microbiome present in salad products.

Methods: Three commercial salad products (A, B, and C) were stored in the refrigerator (4°C) for 15 days. Samplings were on day 0, 5, 10, and 15. PCR-DGGE was used to evaluate the changes of the microbiome in three brands of salad. The Shannon index was calculated to measure microbial diversity. The populations of aerobic and anaerobic bacteria were enumerated by plating samples on trypticase soy agar (TSA) and anaerobic agar (AA).

Results: The Shannon indexes of microbial diversities were 2.234±0.069, 2.573±0.075, and 2.373±0.053 for brands (A, B, and C) initially. The Shannon index decreased from 2.311 to 1.976 for brand B, while the indexes remained the same for brands A and C from day zero to day 15. Total aerobic bacteria increased by 1.582, 1.790, and 1.630 log CFU/g from 8.048, 8.134, and 8.098 log CFU/g by day five for brands A, B, and C, then decreased to 8.050, 8.201, and 8.472 log CFU/g by day 15. The populations of anaerobic bacteria in the three brands decrease by 0.997, 0.685, and 0.347 log CFU/g during the 15 days of storage. Salad from brand B decayed more than the other two brands.

Significance: Different microbial diversity and abundance were found among the three brands. This observation highlights the importance of further evaluating the microbiome present in salad products.

P3-94 The Effects of Relative Humidity on *Salmonella* Biofilm Production, Quorum Sensing, and Subsequent Survival on Tomatoes and Plastic Mulch

JENNIFER TODD-SEARLE[1] and Donald W. Schaffner[2]
[1]*Rutgers University, New Brunswick, NJ,* [2]*Rutgers University, Department of Food Science, New Brunswick, NJ*

Introduction: Tomatoes have been the source of many *Salmonella* outbreaks in the United States, but little is known about the environmental and physiological factors that influence the survival of *Salmonella* on tomatoes and plastic mulch.

Purpose:This project seeked to understand how in vitro *Salmonella* biofilm production and survival on tomatoes and plastic mulch is affected by relative humidity and explained by *Salmonella* rdar (red, dry, and rough) morphology and quorum sensing.

Methods: Desiccators with salt slurries (lithium chloride, potassium carbonate, and potassium sulfate) were used to create controlled RH environments (~15, 50, and 100% RH). Biofilm production of six *Salmonella* strains were tested in microtiter plates with crystal violet. *Salmonella* strains were selected for survival and biofilm studies on tomatoes and plastic mulch. *Salmonella* samples from tomatoes at 100% RH were tested for quorum sensing.

Results: MAE110 had the most and J1890 had the least amount of biofilm production in all RH environments. MAE119 had the largest variability in biofilm production across RH. *Salmonella* strain MAE110 had the rdar morphotype, while other strains did not. MAE110, MAE119, and J1890 were chosen for further testing based on their varying biofilm production and rdar morphotypes. These *Salmonella* strains had the best survival at 100% RH at the end of 14 days on plastic mulch (4.2±0.4 to 6.0±0.7 log CFU/square) and seven days on tomatoes (5.0±0.9 to 7.1±0.2 log CFU/tomato). No biofilm production was observed on plastic mulch at 100% RH. MAE110 was the only strain to show potential biofilm production on tomatoes. Quorum sensing was not observed for any of the three *Salmonella* strains on tomatoes stored at 100% RH.

Significance: Better understanding of *Salmonella* survival on tomatoes could lead to better practices and a reduction in outbreaks. Storing tomatoes at a lower RH resulted in less *Salmonella* survival on tomatoes.

P3-95 Survival of *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* on the Skin and Sliced Kiwifruit

JING YUAN and Luxin Wang
Auburn University, Auburn, AL

❖ ***Developing Scientist Competitor***

Introduction: Pathogens, such as *Escherichia coli* O157:H7 and *Salmonella*, have shown their capability to survive in low pH fruits such as pineapples, mangoes, and strawberries. However, no study has been done for kiwifruit. Two varieties of kiwifruit are available on the market now for consumers. One is the green-fleshed traditional kiwifruit and the other is the yellow-fleshed golden kiwifruit.

Purpose: The objective of this study is to evaluate the survival of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* cocktails on the skin and on sliced kiwifruit.

Methods: The green and golden kiwifruit were purchased from a local grocery store. They were proven to be *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* negative before inoculation following the FDA standard protocols. The whole kiwifruit were inoculated by dipping the entire fruit into the cocktail broth for 30 s then drying and storing them at room temperature for 30 days. The sliced kiwifruit were inoculated by adding 100 µl of each cocktail to the cut surface of every slice. The sliced inoculated kiwifruit were dried and stored at 4°C for 48 h. Selective agars were used to enumerate survived pathogens.

Results: *Escherichia coli* O157:H7 survived for 30 days on whole green kiwifruit while it survived only for 20 days on the golden kiwifruit. The numbers of *Salmonella* and *L. monocytogenes* were below the limit of detection after 15 days on green kiwifruit and 10 days on golden kiwifruit. The pH of cut kiwifruit was 3.48 for golden kiwifruit and 3.51 for green kiwifruit. Although the pH of cut kiwifruit was low, three pathogens survived for 48 hours in the refrigerator.

Significance: This study highlights the importance of preventing contamination of pathogens on raw kiwifruit. Implementation of intervention and preventive control strategies is essential to the safety of fresh cut fruits.

P3-96 Evaluation of *Escherichia coli* Levels Present in Agricultural Ponds and Streams

JING YUAN, Amanda Windham, Kenneth Macklin, Stuart Price and Luxin Wang
Auburn University, Auburn, AL

❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* has been used as an indicator microorganism in water systems. For farms that are under the new produce safety rules, microbiological water quality profiles (MWQP) need to be established when using a surface water resource for irrigation.

Purpose: The objective of this study is to evaluate the microbial quality of agriculture water resources in Alabama and investigate the impact generated by animal agriculture on water quality.

Methods: Water samples and sediment samples were collected from six on-farm ponds located in east central Alabama. These farms differ in size and have different combinations of livestock. Four streams were sampled at the entrance and exit points of each farm to evaluate the impact of animal agriculture. Water samples were processed and plated using the USEPA method 1603 and 3M™ Coliform/*E. coli* Petrifilms. Sediment samples were homogenized with autoclaved MilliQ water and the suspensions were then processed and plated following the USEPA method 1603.

Results: Results show that sampling times (months and seasons) and sample types (sediment vs. surface water) significantly impacted the *E. coli* concentration ($P<0.05$). Sediment samples contained higher numbers of *E. coli* than surface water. *Escherichia coli* counts were higher in summer months. Although *E. coli* concentrations were different between the entrance point and the exit point of each stream, there was no clear indication that animal agriculture generated an impact on water quality.

Significance: The results of this study highlight the importance of establishing MWQP for surface water. Sampling techniques for surface water need to be standardized to avoid impacts generated from the sediment.

P3-97 Microbial Community Analysis of Field Grown Produce in Soil Amended with Manure or Compost from Antibiotic Treated Cattle

KENDALL FOGLER[1], Monica Ponder[1], Amy Pruden[1], Giselle Kristi Guron[1], Leigh Anne Krometis[2], Cully Hession[2], Lauren Wind[2] and Kyle Jacobs[2]

[1]Virginia Tech, Blacksburg, VA, [2]Virginia Tech, Biological Systems Engineering, Blacksburg, VA

❖ ***Developing Scientist Competitor***

Introduction: Cattle are commonly treated with antibiotics that may survive digestion and promote antibiotic resistance in the environment. Manure or composted manure is commonly used as a soil amendment for crop production. Research is needed to determine how prior antibiotic administration and manure management practices influence the microbiome composition of resulting crops, particularly vegetables eaten raw.

Purpose: This study was conducted to determine the effects of antibiotic administration and soil amendment practices on antibiotic resistance and microbial diversity of the edible surfaces of lettuce and radishes grown using recommended application rates.

Methods: Lettuce and radishes were planted in field plots amended with raw manure from antibiotic-treated dairy cows, compost from cows with different histories of antibiotic administration, or no amendment at recommended application rates (12 plots, n=3). Lettuce and radishes were harvested at maturity and the bacterial communities of the edible surfaces compared for each treatment using culture and non-culture based methods. Bacterial log CFU/ml were determined on R2A plates containing seven different antibiotics. DNA was extracted from the surface of mature radish taproots and lettuce leaves. Illumina sequencing of 16S rDNA amplicons, 515F–926R, was performed via Earth Microbiome Project protocol.

Results: Overall the type of soil amendment was not associated with significant differences in total culturable bacteria recovered from the radish taproots or lettuce phyllosphere plated on R2A or on R2A supplemented with the majority of antibiotics. However, bacteria resistant to clindamycin were more numerous on lettuce leaves grown in the soil amendments compared to the fertilizer control (P<0.01). Bacterial diversity (richness, evenness) was influenced by vegetable type, but not soil amendment or antibiotic history.

Significance: Analysis of shot-gun metagenomics sequencing data is underway and used to determine relationships between phylogeny and the resistome. This study will help inform management practices for limiting the dissemination of antimicrobial resistance from farm-to-fork.

P3-98 Dynamics of Bacterial Community on Spinach during Processing and Storage

GANYU GU[1], Samantha Bolten[1], Lan Wang[1], Steve Rideout[2] and Xiangwu Nou[1]

[1]U.S. Department of Agriculture–ARS, Beltsville, MD, [2]Virginia Tech - Eastern Shore AREC, Painter, VA

Introduction: Fresh produce, like spinach, is a known vehicle for a number of recent foodborne outbreaks. Fresh produce harbors large and diverse bacterial communities, potentially including pathogenic bacteria, and the survival and proliferation of bacterial pathogens are strongly influenced by the produce microbiota. However, the microbial ecology of the diverse community on vegetables during processing and storage periods is not well studied.

Purpose: To investigate the shift of bacterial community on spinach during washing/packaging and storage processes.

Methods: Fresh spinach was collected from a commercial fresh produce processor before and after washing/packaging process in the factory. Washed spinach was stored at 4, 10, and 15°C for one week. Bacteria were recovered from unwashed, washed, and stored spinach samples. The population of live bacteria on each sample was evaluated by both plate counting using M9, TSA (with polysorbate 80 and lecithin), and PCA (vegitone) media and quantitative real-time PCR (qPCR) after PMA treatment. The microbiota on spinach samples were analyzed by 16S rRNA sequencing using MiSeq and Qiime2.

Results: Plate-counting results indicated bacterial population on spinach reduced from 6.08±0.13 to 4.91±0.04 log CFU/g after washing process in the tested factory. The population on washed samples increased to 6.74±0.05, 7.1±0.04, and 7.48±0.01 log CFU/g after one week storage at 4, 10, and 15°C, respectively. The estimated bacterial population by qPCR analysis was higher (0.2 to 1 log CFU/g) than plate counting, but followed the same trend. The composition and abundance of bacterial communities on spinach were shifted after washing process and affected by the storage temperature.

Significance: This study provides knowledge on the microbial ecology of diverse bacterial community on spinach during production, which can benefit further studies on the interaction of microbes on produce, and the prevention of foodborne pathogens and spoilage.

P3-99 Fate of *Listeria monocytogenes* on Fresh Apples under Different Storage Temperatures

LINA SHENG[1], Katheryn Edwards[1], Hsieh-Chin Tsai[1], Shima Bibi[1], Ines Hanrahan[2] and Meijun Zhu[1]

[1]Washington State University, Pullman, WA, [2]Tree Fruit Research Commission, Wenatchee, WA

Introduction: *Listeria monocytogenes* was reported to grow on caramel-coated apples during storage. Fresh apples are typically stored for a long period commercially; different apple varieties require dedicated storage temperatures to maintain quality characteristics. There is sparse information about its fate on fresh apples under various storage temperatures.

Purpose: To comprehensively evaluate survival of *L. monocytogenes* on fresh apples at recommended and abusive storage temperatures over a three-month storage period.

Methods: Unwaxed apple fruits of selected varieties (organic Granny Smith apples (GSA), conventional GSA, and Fuji apples) were dip inoculated in a three-strain *L. monocytogenes* cocktail. Inoculated apples (24 h postinoculation, ~6.0, 5.0, or 4.0 log CFU *L. monocytogenes*/apple) were subjected to 1, 4, 10, or 22°C storage for up to three months. Apples under the different storage treatments were sampled at one and four days, and one, two, four, eight, and 12 weeks for survivor enumeration.

Results: During the two-week short-term storage, *L. monocytogenes* population on fresh GSA apples stored at 1, 4, or 10°C remained stable. Reduced *L. monocytogenes* population was observed when GSA were stored at 22°C; there was 0.5~1.5-log CFU/apple reduction 14-day post storage depending on initial inoculation level. *Listeria monocytogenes* showed a similar survival trend on GSA apples during 12-week cold storage under 1, 4, and 10°C; there was 0.5~1.5-log reduction (for both high and low inoculations) with apples in 1°C storage conditions, which showed the least reduction while 10°C showed the most reduction. Furthermore, *L. monocytogenes* demonstrated a similar survival curve on GSA (organic or conventional) and Fuji apples.

Significance: A limited reduction of *L. monocytogenes* on apple surfaces occurred during 12 weeks of refrigerated storage. This data will provide industry with important information to assess the potential risk of *L. monocytogenes* on fresh apples.

P3-100 Survival of *Listeria innocua* on Fuji Apples under Commercial Cold Storage with or without Ozone Gaseous

LINA SHENG[1], Ines Hanrahan[2], Xiaofei Sun[1], Michael Taylor[1], Glade Brosi[3] and Meijun Zhu[1]
[1]*Washington State University, Pullman, WA,* [2]*Tree Fruit Research Commission, Wenatchee, WA,* [3]*Stemilt Growers LLC, Wenatchee, WA*

Introduction: *Listeria monocytogenes* was linked to a caramel apple outbreak and reported to grow on caramel-coated apples during storage. Ozone, an FDA approved GRAS, has broad antimicrobial effect but no residual effects. However, there is only limited knowledge and information about the fate of *Listeria* on fresh apples during commercial cold storage and the efficacy of continuous low doses of gaseous ozone application during the cold storage in controlling *Listeria* on apples.

Purpose: This study examined the fate of *Listeria innocua* on apples during commercial cold storage to further evaluate antimicrobial efficacy of low dose gaseous ozone against *L. innocua*.

Methods: Unwaxed Fuji apples were dip inoculated in a three-strain *L. innocua* cocktail. Inoculated apples (24h postinoculation, 6.0 log CFU *L. innocua/*apple) were subjected to refrigerated atmosphere (RA), controlled atmosphere (CA), or CA with low doses of ozone during storage in a commercial facility. Survivor enumeration occurred at 1-, 3-, 6-, and 12-weeks. Simultaneously, a set of uninoculated Fuji apples were subjected to aforementioned storage treatments and sampled every six weeks for total bacterial count (TPC) and yeast/mold count (Y/M).

Results: Commercial cold storage (RA and CA) had little effect on TPC, but led to ~ two-log reduction of *L. innocua* on apples over 12-weeks of storage. *Listeria* survival and TPC count of apples under RA or CA are similar; Y/M was slightly decreased on apples under CA storage compared to the RA storage. Low dose gaseous ozone application enhanced *L. innocua* reduction, resulting in an additional one to two-log reduction over 12-weeks storage; reduced TPC and Y/M by ~ 0.5 and 0.7 log during 12-weeks storage, respectively.

Significance: Low dose gaseous ozone application in commercial CA storage had a positive effect on fruit microbial safety against *Listeria* on apples, as well as on fruit decay.

P3-101 Survival of Generic *E. coli* on Gala and Golden Delicious Apples Near Harvest with and without the Use of Overhead Cooling Water Applications

Kyu Ho Jeong[1], **INES HANRAHAN**[2], Lauren Walter[1], Meijun Zhu[1] and Karen M. Killinger[1]
[1]*Washington State University, Pullman, WA,* [2]*Tree Fruit Research Commission, Wenatchee, WA*

Introduction: Overhead evaporative cooling (EC) using surface water is frequently used in Washington to decrease sunburn in apples to prevent economic losses, but influence on food safety risk is uncertain.

Purpose: Reduction of inoculated generic *E. coli*levels was evaluated on Gala and Golden Delicious apple varieties with and without EC water application for up to one week.

Methods: A four-strain, rifampicin-resistant generic *E. coli* cocktail was inoculated onto apples using a backpack sprayer after sunset on selected varieties (Gala and Golden Delicious) with or without EC in replicated blocks and harvested from at least two canopy positions (high, low) in a three year study. Apples were sampled at 0, 2, 10, 18, 34, 42, 58, 82, 106, and 154 hours after inoculation. Survivors on apples were enumerated on Chromagar ECC-rifampicin with and without filtration; pre-enrichment in TSB was performed as generic *E. coli* levels declined. Uninoculated control apples were tested for indicator organisms as generic *E. coli.*

Results: Initial inoculum levels on apples averaged 7.3-7.4 log CFU/apple. Generally, the greatest reduction in generic *E. coli* levels was observed within the first 8-10 hours after inoculation, with additional reduction at a slower rate between 34-106 hours. Averaged over three years, at 10 hours after inoculation, generic *E. coli* was reduced 2.1 and 2.5 log CFU/apple for untreated Gala and Golden Delicious, respectively and 2.8-2.9 log CFU/apple for EC treated fruit. The reduction of generic *E. coli* varied dramatically among individual apples within the same variety at any given time point. For uninoculated control apples, 3 out of 180 apples had detectable levels of generic *E. coli*.

Significance: Treatment with EC did not appear to enhance survival of generic *E. coli* on apples compared to the response on control apples that did not receive EC application.

P3-102 *Arcobacter* Internalization in Fresh Produce: An Emerging Food Safety Issue under Extreme Weather Events

SEUNGJUN LEE
The Ohio State University, Columbus, OH

Introduction: The *Arcobacter* is an emerging waterborne and foodborne enteric pathogen. *Arcobacter* internalization is an important issue in raw vegetable consumption because washing and chlorination usually cannot remove the internalized pathogens effectively.

Purpose: The objectives of this study were to 1) determine the extent of *Arcobacter* internalization into plants through roots or leaves; and 2) investigate the impact of extreme weather events, drought and heavy rains, caused by climate change.

Methods: *A. butzleri* was selected since it is a serious hazard to human health. Iceberg and romaine lettuce were cultivated, underwent three conditions (optimal, drought, storm) and contaminated with 10^7, 10^8 and 10^9 colony forming unit (CFU)/g soil.

Results: Results show that *A. butzleri* were internalized via entering the leaf surfaces and roots under all the weather conditions. Under the optimal condition (25–30°C, 50–60% of relative humidity), a dose-dependency was observed. Under extreme conditions (drought and storm), the internalization level was higher than the lettuce grown at the optimal condition. Especially, under drought, the internalization level increased by 11 times and 20 times compared to the optimally irrigated group when the soil was contaminated with 8 and 9 log CFU/g soil, respectively.

Significance: In summary, *A. butzleri* accumulate inside the lettuce from the contaminated soils and stay in the edible parts. The internalization levels were different depending on the location (leaf, root). The extreme weather conditions (drought or storm) can increase the level of internalization of *A. butzleri*.

P3-103 Cross-contamination by Curli and Non-curli Producing *Escherichia coli* O157:H7 Mediated by Lettuce Inoculation Location during Washing of Leafy Greens

ANN VEGDAHL[1] and Donald W. Schaffner[2]
[1]*Rutgers University, New Brunswick, NJ,* [2]*Rutgers University, Department of Food Science, New Brunswick, NJ*

❖ ***Developing Scientist Competitor***

Introduction: Curli are thin, adhesive structures on the surface of *Escherichia coli* cells and may have a role in pathogenesis. Curli can mediate attachment of *E. coli* to various biotic and abiotic surfaces, internalization into intestinal cells, and formation of biofilms.

Purpose: The objective of this study was to determine how curli affect cross-contamination of *E. coli* during washing from inoculated to non-inoculated lettuce pieces considering different inoculation locations.

Methods: Overnight cultures of *E. coli* O157:H7 strain 0018 (± for curli) were centrifuged and resuspended in peptone water. A single piece of baby romaine lettuce was spot inoculated either on the surface, freshly cut edges, or both the surface and the edge with approximately six log CFU. Leaves were dried for 2 hours, and washed with 10 uninoculated pieces in a stainless-steel bowl for 30 seconds. Bacterial reduction and cross-contamination were quantified. Data were plotted and analyzed using Microsoft Excel and Statplus.

Results: Fewer curli producing *E. coli* transferred to water than non-curli producing *E. coli* ($P<0.05$). The percent transfer to water ranged between 80-99% when *E. coli* was inoculated on the lettuce surface and 24-80% when the cut-edges were inoculated. Log reduction from washing was higher for the non-curli producing *E. coli* and significantly different when the lettuce surface was inoculated ($P=0.01$). There was no significant difference in washing log reduction for curli and non-curli producers when the cut-edges were inoculated ($P=0.42$). The transfer to uninoculated pieces was significantly less for the non-curli strain than curli producing strain, regardless of the inoculation location ($P<0.01$).

Significance: The *E. coli* O157:H7 strain lacking curli more readily transferred to the water, but appeared to lack the ability to readily reattach to lettuce leaves. These findings suggest that targeting curli might be an effective means of combating *E. coli* transfer during washing of leafy greens.

P3-104 *E. coli*/Salad Interactions

GIANNIS KOUKKIDIS and Primrose Freestone
University of Leicester, Leicester, United Kingdom

❖ ***Developing Scientist Competitor***

Introduction: Fresh produce such as salad leaves are an important part of a healthy diet but in recent years have been associated with infection by enteric pathogens such as *E. coli* and *Salmonella enterica*. In July 2016 a UK *E. coli*-rocket outbreak made>150 people ill and killed 2. However, despite calls for improvements in salad leaf hygiene until our recent publication, few studies had investigated enteric pathogen behaviour when actually within a bagged salad. Salad leaves become damaged during processing and juices are released, so bacteria residing in a salad bag will be bathed in leaf juice.

Purpose: The intention of our research is to investigate the effect of juices released from damaged salad leaves on the growth, virulence and salad leaf colonisation of *E. coli*. Our aim is to use this information to develop ways of preventing enteric pathogen attachment to fresh salad produce.

Methods: *E. coli* responsiveness to salad juices was analysed in water, to reflect the salad bag environment, and in more host-like serum- media to model the co-consumption of pathogen and salad leaf. We used assays that measured the effect of salad leaf juice on *E. coli* growth, motility and biofilm formation. Light and scanning electron microscopy were used to visualise juice effects on *E. coli* colonisation of salad leaves and the salad container.

Results: Salad juices at>1/50 dilutions significantly stimulated *E. coli* growth in all media tested. In serum-media, juices enhanced growth by several logs via provision of host iron from serum-transferrin. In water, leaf juices from all salad leaves as well as the fluid within the bag salad tested significantly increased *E. coli* biofilm formation and its capacity to colonise and persist on salad leaves, and the salad bag container.

Significance: Our study shows that even very dilute salad juice can contribute to *E. coli* colonisation of salad leaves and re-emphasises the importance of preventing enteric pathogen of fresh produce.

P3-105 Microbial Analysis of Produce Purchased from Florida Farmers' Markets

LISA ROTH, Amy Simonne, Lisa House and Soohyoun Ahn
University of Florida, Gainesville, FL

Introduction: The number of U.S farmers' markets has increased by 72% since 2010, which can be attributed to the consumer demand for locally grown fresh produce. However, data on the microbial quality of produce sold at farmers' markets in Florida is lacking.

Purpose: The purpose of this study was to determine the prevalence of indicator organisms and pathogens in fresh produce from Florida farmers' markets.

Methods: A total of 179 fresh produce samples (72 tomatoes, 60 leafy greens, 22 berries, and 25 spinach) were collected from 9 farmers' markets in North and Central Florida. Total coliforms and generic *E. coli* were analyzed using 3M *E. coli*/Coliform Count plates. *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7 were analyzed by real-time PCR assay kits.

Results: Sixty eight percent ($n=107$) of produce samples were positive for total coliforms. Lettuce and spinach had the highest percent of samples with coliforms (84.3% and 84.2%, respectively), followed by tomatoes (65.7%) and berries (19%). Generic *E. coli* was detected in 1.27% ($n=2$) of samples. Two samples (1.12%) were positive for *L. monocytogenes* and no *Salmonella* and *E. coli* O157:H7 were detected.

Significance: The data from this study provides useful information on the microbiological quality of fresh produce from farmers' markets in Florida and may aid in the development of food safety programs for farmers' markets or direct-to-consumer sales outlets.

P3-106 Influence of Ultraviolet-C Light Intensity on Blueberries for Enhanced Food Safety and Extended Shelf Life

AROSHA LOKU UMAGILIYAGE[1] and Ruplal Choudhary[2]
[1]*Southern Illinois University, Carbondale, IL,* [2]*Southern Illinois University - Carbondale, carbondale, IL*

Introduction: As one of the most popular fruits, blueberries have many benefits to human health. However, the highly perishable nature of the blueberry leads to short shelf life; the fruit is easily spoiled by mold. In addition, possible contamination of blueberries, which can occur during agronomic practices and post-harvest handling, leads to human food safety issues.

Purpose: The research assessed the effect of UV-C intensity on total yeast and mold count, bacterial reduction, blueberry quality, and shelf-life.

Methods: Blueberries were exposed to three different radiation intensities of low=1.1 mW/m^2; medium 4.1 mW/m^2 and high=7.6 mW/m^2 as treatment and those intensities were applied separately at three different doses (1, 2, and 4 kJ/m^2) as an experimental block. By the time the treatments were completed, fifty blueberries were packed in groups, in plastic clamshells, and stored 4◦C. Untreated blueberries were packed and stored, as previously described, as controls. At each sampling time of 0, 1, 7, 14, 21, 28, 35, 49, and 70 days, berries were immediately analyzed or freeze dried.

Results: Gray mold (*Botrytis cinerea*) was the main causal agent for decaying fruit. Weight loss, titratable acidity, soluble solids, and firmness were not significantly affected by UV-C intensity. UV-C treatment reduced decay, significantly, and fruit exposed to higher intensities showed significantly lower mold deterioration (P<0.05). Mold count were not affected by increased intensity, suggesting that decrease mold decay did not result from greater germicidal effect of UV-C, but some indirect mechanism mediated improving defense response of fruit by increased intensity. Total anthocyanin content and total phenolic content values increased with treatment intensity.

Significance: UV-C treatment reduced storage loss by one third at the end of 10 weeks. For a given dose, increasing UV-C intensity increased fruit quality and reduced spoilage loss, while significantly reducing the treatments time.

P3-107 Characterization of *Clostridium difficile* Isolated from Animal Manure and Manure-based Compost

MUTHU DHARMASENA and Xiuping Jiang
Clemson University, Clemson, SC

❖ ***Developing Scientist Competitor***

Introduction: *Clostridium difficile* has been recognized as a community associated pathogen. Previous studies have reported that food producing animals carry and shed this pathogen. Therefore, animal manure-based compost may play a role in disseminating toxigenic *C. difficile* in agricultural environments.

Purpose: The objective of this study was to isolate *C. difficile* from animal manure and commercial compost samples and characterize their toxigenicity.

Methods: Commercial compost and fresh manure samples (n=142) were enriched in brain heart infusion broth supplemented with 0.5% yeast extract, 0.1% L-cysteine, 0.1% sodium taurocholate, and antibiotics (moxalactam and norfloxacin) (BHIB-YE-CYS-T-MN) for 7 days, anaerobically, and recovered on *C. difficile* agar base supplemented with 7% horse blood, 0.1% L-cysteine, 0.1% sodium taurocholate, and antibiotics (moxalactam and norfloxacin). Suspected colonies were confirmed as *C. difficile* by PRO disc method, PCR for *tpi*, and latex agglutination tests. Isolates were tested for toxigenic genes, *tcdA*, *tcdB*, *cdtA*, and *cdtB*, and further identified by capillary gel electrophoresis.

Results: Samples were categorized as cow manure-based, poultry litter-based, mix manure-based, biosolid waste-based, plant-based, and other. A total of 58 *C. difficile* isolates were detected from 51 samples (47 finished compost and 4 manure). The majority of the isolates were toxigenic (67%), but binary toxins were observed only in 3 (5%) isolates. There were 45 different PCR-ribotypes among the 58 *C. difficile* isolates. The highest diversity in ribotypes was observed among the *C. difficile* isolates from biosolid waste-based composts. The most common toxigenic ribotype was RT500, and other more prevalent ribotypes were RT020, RT412, and RT251. RT009 was found as the most prevalent non-toxigenic ribotype.

Significance: The presence of toxigenic *C. difficile* ribotypes in finished compost suggests the contaminated compost/manure-amended soil could serve as a potential reservoir for *C. difficile* that may contaminate fresh produce, water, or field workers on the farm.

P3-108 Changes in the Phyllosphere-associated Bacteria of Leafy Greens Caused by Environmental Factors Such as Solar Radiation

PILAR TRUCHADO, Maria Gil and Ana Allende
CEBAS-CSIC, Espinardo, Spain

Introduction: The phyllosphere associated bacteria represents a dynamic population in constant change, probably affected by extrinsic factors such as agricultural practices and climatic conditions. Among climatic factors, solar radiation has been highlighted as a major factor affecting bacterial colonization and survival. However, the impact of solar radiation on specific bacteria phyla of leafy greens has not been addressed.

Purpose: This study was conducted to determine the effect of solar radiation on the bacterial community of red-pigmented baby leaf lettuce using cultivation dependent and independent techniques.

Methods: Baby lettuce, grown in open field, were subjected to two different solar radiation intensities using a light-excluding plastic mulch to reduce the sunlight exposure. Four weeks before harvest, the field was divided in two plots; one of them was covered, while the other was kept uncovered. Photosynthetically active radiation (PAR) was measured weekly and the cumulative PAR was calculated to distinguish the treatments as 4265±356 µmol/m^2/s (uncovered) and 3115±313 µmol/m^2/s (covered). The impact of the solar radiation on the bacterial communities was quantified using qPCR primers for the 16S ribosomal sequences of the most predominant phyla, including *Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria, Bacteroidetes, Actinobacterias* and *Firmicutes,* as as well as total bacteria.

Results: The total bacterial population size was not affected by solar radiation treatments. However, significant differences were observed in the relative abundance of bacterial community between treatments. In general, solar radiation reduced the relative abundance of *Gammaproteobacteria,* while it increased the relative abundance of *Betaproteobacteria*. Obtained results, also, confirmed that *Proteobacteria*, *Bacteroidetes*, *Actinobacterias* and *Firmicutes* were the most abundant phyla in baby pigmented lettuce.

Significance: This study described, for the first time, the impact of the solar radiation on specific groups mong the phyllosphere-associated bacteria in pigmented baby lettuce. The obtained results are relevant, but should be completed to determine if the changes on the relative abundance of specific phyla might have an impact on the safety of leafy greens.

P3-109 Microbial Quality of Irrigated Leafy Green Vegetables in Accra, Ghana

JOYCELYN K. QUANSAH[1], Angela P.H. Kunadu[2], Lydia Mosi[3], Firibu K. Saalia[2] and Jinru Chen[1]
[1]University of Georgia, Department of Food Science and Technology, Griffin, GA, [2]University of Ghana, Department of Nutrition and Food Science, Accra, Ghana, [3]University of Ghana, Department of Biochemistry, Cell and Molecular Biology, Accra, Ghana

Introduction: Vegetable farming in urban areas of Ghana have increased due to increasing market demands, despite the scarcity of land and water. Most famers have situated their farms close to various water sources for irrigation.

Purpose: This study determined the microbial quality of some leafy green vegetables from irrigated vegetable farms in selected urban areas of Accra, Ghana.

Methods: Two exotic (lettuce and cabbage) and four indigenous (*Amaranthus* sp., *Solanum macrocarpon, Hibiscus sabdariffa*, and *Corchorus olitorius*) leafy green vegetables (n=175) were collected in duplicate from 50 farms in 12 farming areas in urban areas of Accra, Ghana from March to December,

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2016. The vegetables rinses (0.1M phosphate buffered saline) were inoculated onto tryptic soy agar, MacConkey agar, Enterococcus agar, and potato dextrose agar and resulting microbial colonies were enumerated. Presumptive *Salmonella* colonies were isolated using the FDA BAM protocol.

Results: Average total aerobic bacteria, yeast and mold, fecal coliform, and enterococcus counts on collected vegetables ranged from 8.20 to 9.65, 4.22 to 5.20, 3.96 to 5.02 and 2.71 to 3.13 log CFU/g, respectively. Exactly 143 vegetables from 48 farms tested positive for fecal coliforms and 136 vegetables from 48 farms tested positive for enterococcus. Seven vegetable samples tested positive for presumptive *Salmonella*. Farming area and vegetable type had significant influence ($p \leq 0.05$) on average aerobic bacteria and yeast and mold counts. Farming area and farm had a significant influence ($p \leq 0.05$) on fecal coliform and enterococus counts. Cabbages had the highest aerobic bacteria counts, whilst lettuce had the lowest. The indigenous vegetables were higher in yeast and mold counts than cabbage and lettuce. *Amaranthus* sp. and cabbage had the lowest fecal coliform counts.

Significance: The results of this study suggested that leafy green vegetables cultivated in urban areas of Ghana were associated with high microbial counts. Consumption of fresh leafy green vegetables without sanitizing or heat treatment should be discouraged.

P3-110 New Culture Media Method, Approved by AOAC and Microval for Rapid and Convenient Detection and Enumeration of Food Spoilage Microorganisms

ANKE HOSSFELD[1], Renaud Chollet[2], Lisa John[1] and Celine Marion[2]

[1]Merck KGaA, Darmstadt, Germany, [2]Millipore SAS, Molsheim, France

Introduction: The Food and Beverage Industry needs methods to reduce workflow and provide reliable, faster results compared to traditional culture media. Convenient culture medium devices are developed for rapid and improved detection of Total Aerobic Count, *Escherichia coli*, Coliforms, Yeasts and Molds. Devices comprise dried culture medium coated onto a pad protected by a transparent lid. 1ml food sample inoculates and rehydrates, according to reference method sample preparation procedures. Enumeration follows 24h to 72h incubation. Color-indicator and coding enables easy differentiation and improved read-out.

Purpose: To pre-evaluate convenient media device performance for microbial monitoring in two key applications: Dairy and Processed Food. Total Aerobic Count, Coliforms, *E. coli*/Coliforms and Yeasts/Molds were assessed.

Methods: 7 microorganisms were artificially inoculated into 2 food matrices: pasteurized chocolate milk and canned tomato, at 10 to 100 CFU per test, in triplicate. Growth performance and enumeration were evaluated against ISO reference methods. Sample preparation followed the appropriate reference method, except inoculation volume: 1mL.

Results: *Cronobacter sakazakii* and *Lactobacillus plantarum* were detected respectively within 24h and 48h at 35°C, in both matrices, with the Total Aerobic Count device. Enumeration was comparable to the ISO 4832 and ISO 4833 reference methods and counting was facilitated by the universal red color of colonies. *E. coli*, *Enterobacter cloacae* and a mixture of both were detected by coliform and E.coli/coliform devices within 24h at 35°C in both matrices. The E.coli/coliform device differentiated by color *E. coli* colonies (red-purple) from coliform (blue). *Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus brasiliensis*were detected in 48h at 25°C with the Yeast/Mold device with comparable results to the ISO 6611:2004 reference method.

Significance: Convenient culture media devices are a reliable alternative method to reduce time and workload in food spoilage detection.

P3-111 Withdrawn

P3-112 Withdrawn

P3-113 Withdrawn

P3-114 A Study to Assess the Numbers and Prevalence of *Bacillus cereus* and Its Toxins in Pasteurized Fluid Milk

SALEEMA SALEH-LAKHA[1], Carlos Leon-Velarde[1], Shu Chen[1], Susan Lee[1], Kelly Shannon[1], Martha Fabri[2], Bruce Keown[2] and Gavin Downing[2]

[1]University of Guelph, Guelph, ON, Canada, [2]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada

Introduction: *Bacillus cereus* is a pathogenic adulterant of raw milk and can persist as spores and grow in pasteurized milk. Foodborne outbreaks attributed to *B. cereus* toxins may be under-reported due to short duration of the illness and potential misdiagnosis with diseases caused by *Staphylococcus aureus* or *Clostridium perfringens*.

Purpose: The objectives of this study were to determine the prevalence of *B. cereus* and its enterotoxins in pasteurized milk at its best-before date and to conduct toxigenic characterization of *B. cereus* isolated from milk.

Methods: *Bacillus cereus* counts in milk samples (n = 254) stored at 4°C, 7°C, and 10°C were estimated according to MFLP-42, "Isolation and Enumeration of the *Bacillus cereus* group in Foods". Samples containing >10^4cfu *B. cereus*/ml were analyzed for the presence of enterotoxins using the 3M™ TECRA™ Bacillus Diarrhoeal Enterotoxin Immunoassay. *Bacillus cereus* isolates obtained from milk were analyzed for the presence of toxin genes *nheA*, *entFM*, *hblC*, *cytK* and *CER* via multiplex PCR.

Results: Over 5.5% of moderately temperature-abused products (stored at 7°C) were found to contain >10^5 cfu *B. cereus*/ml and 4% contained enterotoxins. Over 31% of products contained >10^5 cfu of *B. cereus*/ml and associated enterotoxins when stored at 10°C. Enterotoxin production by *B. cereus* in pasteurized milk started at 7-8 days of 7°C storage. Characterization of 17 representative *B. cereus* isolates from pasteurized milk revealed five toxigenic gene patterns, with all of the strains carrying genes encoding for diarrhoeal toxins, but not for an emetic toxin. One of the strains contained all four diarrhoeal enterotoxin genes (*nheA*, *entFM*, *hblC*, and *cytK*).

Significance: The results of this study demonstrated the risks associated with moderately temperature-abused, pasteurized milk and the necessity of a controlled cold chain throughout the shelf life of fluid milk in order to enhance product safety and minimize foodborne illness.

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P3-115 Evaluation of a Highly Multiplexed, Automated Assay for the Detection of Beer Spoilage Flora

MORGAN WALLACE, Daniela Bocioaga, Kathryn Mix, Stephanie Morse, Kyleen Sorensen, Cristina McGuire, Sarah Kozak, Jessica Pecone, Katherine Sweitzer, Peter Trabold and Gwendolyn Spizz
Rheonix, Ithaca, NY

Introduction: Organisms that can spoil beer include both yeast and bacterial strains. Culture-based isolation and identification may require lengthy procedures with skilled interpretation. Rapid, non-culture methods, such as PCR, can provide fast and accurate detection. These methods shorten the overall time to result from more than five days to a single day. The Rheonix SpoilerAlert™ assay kit is highly automated, with only a single pipetting step required to obtain PCR results from a sample. The initial commercial version of the assay amplifies 10 target genes present, individually or in combination, in various lactic acid bacteria and the wild yeast, *Brettanomyces bruxellensis*. The assay also detects the brewers' yeast, *Saccharomyces cerevisiae.* Due to industry concern*, S. cerevisiae* var. *diastaticus* target was added.

Purpose: The purpose of this study was to characterize the performance of the updated assay for detection of spoilage flora and hop resistance genes. Inclusivity and exclusivity of the new assay was characterized and sensitivity was determined.

Methods: Studies evaluating the assay were conducted using target and non-target cells, with and without enrichment.

Results: The sensitivity of the assay was determined to be ~10^4 CFU/ml for each of the targets in post enriched samples and <10 CFU/sample before enrichment. The additional target for *S. cerevisiae* var. *diastaticus* was detected, as expected, in this variant but was not detected in multiple *S. cerevisiae* strains that were not *S. cerevisiae* var. *diastaticus*.

Significance: This method for beer spoilage flora detection and characterization provides reduced processing time compared to culture and provides a genetic characterization of the bacterial genes that confer hop resistance. This allows brewers to make earlier decisions regarding spoilage flora presence in their products and processing environments. This technology can be used for other market segments, where well characterized microbiota are associated with reduced shelf life.

P3-116 Health Canada Validation of a Chromogenic Medium for Enumaration of *E. coli* and other Non-*E. coli* Coliforms Bacteria in Selected Food Matrices

YANNICK BICHOT[1], Wendy Lauer[2], Mike Clark[3] and Christophe Quiring[4]
[1]Bio-Rad, Marnes-la- Coquette, France, [2]Bio-Rad Laboratories, Hercules, CA, [3]Bio-Rad Laboratories, Battlefield, MO, [4]Bio-Rad Laboratories, Steenvoorde, France

Introduction: Food laboratories often rely on the enumeration of Coliform group and/or *Escherichia coli* in food ingredient, finished product, or production environment to indicate the potential contamination of food products by fecal material and/or the potential presence of pathogens.

The RAPID'E.coli 2 protocol allows the enumeration and differentiation of *E. coli* and other Coliform bacteria in a single chromogenic agar plate and in less than 24 hours without any confirmation. The method has been recently validated by Health Canada.

Purpose: The objective of this study is to summarize the performance evaluation did in order to obtain the Health Canada approbation for publication in Compendium of Analytical **Methods** .

Methods: The evaluation has been conducted through two sets of results coming from AOAC Performance-Tested Method 050601 and AFNOR validated protocol BRD 07/08-12/04, BRD 07/07-12/04, BRD 07/01-07/93. The RAPID'E.coli 2 protocol has been compared to the reference method AOAC 966.24, ISO 16649-2 and ISO 4831. The inclusivity was tested using 108 isolates of *E. coli* (60) and non-*E. coli* coliform bacteria (48) and the exclusivity was tested using 32 non-coliform bacteria. A Method Comparison Study was conducted on several food categories using three inoculation levels using naturally contaminated products and artificial contamination. An Inter-Laboratory Study was run involving 10 laboratories.

Results: RAPID'E.coli 2 agar yielded an inclusivity rate of 99% and an exclusivity rate of 94%. For *E. coli*, the critical Level, limit of detection and limit of quantification determined during the method comparison study were 2.97, 4.58 and 12.93 CFU/ml respectively and for other coliforms 3.47, 5.94 and 13.43 CFU/ml respectively.

Significance: RAPID'E.coli 2 was shown to be an effective and efficient method for enumeration of *E. coli* and coliforms in only 24 h.

P3-117 Development of a Monte Carlo Model to Predict Fluid Milk Spoilage by Aerobic Psychrotolerant Sporeformers

ARIEL BUEHLER[1], Aljosa Trmčić[2], Nicole Martin[1], Kathryn Boor[1] and Martin Wiedmann[1]
[1]Cornell University, Ithaca, NY, [2]University of British Columbia, Vancouver, BC, Canada

❖ ***Developing Scientist Competitor***

Introduction: Psychrotolerant sporeforming bacteria represent a major challenge regarding microbial spoilage of fluid milk. These organisms can survive most pasteurization regimes and subsequently germinate and grow to spoilage levels during refrigerated storage.

Purpose: Modeling spoilage of fluid milk due to growth of psychrotolerant sporeforming bacteria will allow for improved predictions of fluid milk shelf life and will facilitate assessment of different approaches to control psychrotolerant sporeforming bacteria in the fluid milk production and processing continuum.

Methods: Spore suspensions of psychrotolerant sporeformers, representing the most common Bacillales subtypes, isolated from raw and pasteurized milk were used to test germination and growth in skim milk broth at 6°C. Complete growth curves were obtained by following total and spore count every 24 h. Based on growth curves at 6°C, probability distributions of initial spore counts in bulk tank raw milk and subtype frequency in bulk tank raw milk, a Monte Carlo simulation model was created to predict spoilage patterns in HTST-pasteurized fluid milk.

Results: Growth parameters lag phase (λ) and maximum growth rate (μ_{max}) were significantly different among psychrotolerant sporeformer subtypes. Lag phase ranged from 1.9 to 18.2 days and maximum growth rate ranged from 0.64 to 1.5 log CFU/mL/day. Given current raw milk sporeformer contamination patterns, Monte Carlo simulations indicated that 91% of samples of HTST fluid milk will have a cell density greater than 20,000 CFU/mL by day 21 of storage at 6°C, consistent with current spoilage patterns observed in commercial products.

Significance: This study provided a baseline understanding of the growth rates of psychrotolerant sporeformers in fluid milk and provided a stochastic model of spoilage by these organisms over the shelf-life of fluid milk, which will allow for assessment of different approaches to reduce fluid milk spoilage.

Poster

P3-118 Patulin Contamination and Patulin-producing *Penicillium* spp. in Decayed Apples and Patulin Reduction by Mechanical Removal of Decayed Parts

MIN JUNG, Sung Min Cho, Min Jung Choo, Kyu Ri Lee, Sung-Yong Hong and Soo-Hyun Chung
Korea University, Department of Integrated Biomedical and Life Science, Seoul, South Korea

Introduction: Patulin is a mycotoxin produced by some species of fungi, particularly *Penicillium expansum,* on apples. It can cause severe acute and chronic toxicity including carcinogenicity and immunotoxicity.

Purpose: This study aimed to investigate the occurrence of patulin contamination and the incidence of patulin-producing *Penicillium* spp. on decayed apples and to determine the effect of mechanical removal of decayed portions on patulin reduction.

Methods: Fungi on 53 decayed apples were isolated on Potato Dextrose Agar plates. To identify the fungal isolates, morphological and molecular examinations (sequencing of ITS1 and 4, and beta-tubulin gene) were carried out. Thirty *Penicillium* sp. were grown on apple agar plates for 7 days at 25°C to evaluate intraspecific variability of growth and patulin production. After incubation with *Penicillium expansum* KUFMNS 21 for 14 days, the decayed parts of apples were scooped out and analyzed for patulin by HPLC-UV (276nm).

Results: A total of 58 fungi were isolated from 41 apples, of which 17 were contaminated with patulin (0.1-2.0 μg/g). Thirty isolates were classified as *Penicillium* spp. (29 *P. expansum,* 1 *Penicillium echinulatum*)*.* Twenty-eight fungi were categorized into genera such as *Alternaria*, *Fusarium*, and *Collectotrichum*. All of the 29 *Penicillium expansum* produced patulin (0.2 μg/g– 1.1 μg/g) on apple agar plates within 7 days. *Penicillium expansum* KUFMNS 21 produced patulin up to 0.8 and 0.7 μg/g on 2 apple cultivars (Royal and Mishima) within 14 days, respectively. Patulin reduction in the apples was 92.9% (Royal) and 95.7% (Mishima) after removal of the decayed parts of apples on the seventh day.

Significance: The majority (41 out of 53) of the decayed apples were contaminated with fungi. Twenty-nine *P. expansum* out of 58 fungal isolates produced patulin. The mechanical removal of decayed apple parts effectively reduce the patulin contamination.

P3-119 A Putative Siderophore Locus of *Pseudomonas fragi*: Solving an Iron Problem

TAMSYN STANBOROUGH[1], P. Scott Chandry[1], Shane M. Powell[2], Mark Tamplin[2] and Narelle Fegan[1]
[1]CSIRO Agriculture & Food, Werribee, Australia, [2]Food Safety Centre, Tasmanian Institute of Agriculture, University of Tasmania, Hobart, Australia

❖ ***Developing Scientist Competitor***

Introduction: *Pseudomonas fragi* is a dominant meat and raw milk spoilage bacterium, which may be attributed to its efficient utilization of the iron present in these foods. Unlike other members of its genus, it is currently thought that *P. fragi* does not synthesize siderophores.

Purpose: The aim of this work was to characterize a putative siderophore locus of *P. fragi* and determine whether strains that possess this region secrete siderophores.

Methods: A putative siderophore locus was identified in 12 *P. fragi* isolates of meat, milk, and non-food origin. Protein homology and domain analysis of locus components was performed, as well as a search for protein binding sites for regulatory proteins. Siderophore production of the strains was tested using Chrome azurol S (CAS) agar and supernatant assays.

Results: A genomic region of approximately 10 kb identified in 12 *P. fragi* strains was found to be homologous to the *xss* operon in xanthomonads, which encodes genes for xanthoferrin (α-hydroxycarboxylate-type siderophore) and xanthoferrin-mediated iron uptake, contributing to growth and virulence of these plant pathogens under iron-limiting conditions. Like the *xss* operon, this region in *P. fragi* comprised seven genes: Five siderophore biosynthesis genes, a TonB-dependent siderophore receptor gene and a membrane spanning transporter gene with homology to multidrug efflux pumps of the major facilitator superfamily. Upstream of this region, a potential Fur binding site was identified, which contained 13/19 residues conserved in the *Xanthomonas campestris* pv. *campestris* Fur-box consensus sequence. Moreover, siderophore production of the strains was confirmed using both CAS agar and supernatant assays.

Significance: Characterization of this locus and the role these genes play in iron metabolism may help us understand how *P. fragi* can compete so efficiently with other meat and milk contaminants and may lead to the development of effective strategies to limit *P. fragi* growth and spoilage of food.

P3-120 Biodegradation of Aflatoxin B_1 by Edible Mushroom Cultures and Their Cell-free Extracts

MIN JUNG CHOO, Kyu Ri Lee, Sung Min Cho, Min Jung, Sung-Yong Hong and Soo-Hyun Chung
Korea University, Department of Integrated Biomedical and Life Science, Seoul, South Korea

❖ ***Developing Scientist Competitor***

Introduction: Aflatoxins are highly toxic secondary metabolites predominantly produced by toxigenic *Aspergillus flavus* and *Aspergillus parasiticus*. Of these aflatoxins, aflatoxin B_1 (AFB_1) is the most potent carcinogen. It has been reported that edible mushrooms including *Pleurotus ostreatus*, have AFB_1 biodegradation activity.

Purpose: The purpose of this study was to investigate the AFB_1-degrading activity of edible mushroom cultures and their cell-free extracts.

Methods: Twenty edible mushrooms in Basidiomycota were obtained from mushroom farms in Gyung-gi province in Korea. AFB_1-degrading activity was screened using potato dextrose broth (PDB) containing 50 ng/mL of AFB_1. To find out AFB_1 degradation mechanism, cell-free extracts and cell-free culture broth were spiked with 0.1 – 1 μg/mL of AFB_1 and incubated at 25°C for 2 days. AFB_1 reduction in the samples was analyzed by HPLC-FLD.

Results: AFB_1 in PDB was reduced by 5 mushrooms; *Auricularia auricular-judae*, *Bjerkandera adusta*, *Hericium erinaceus*, *Lentinula edodes*, and *Poria cocos*. The cell-free extract of the mushrooms showed significant AFB_1-degrading activity; AFB_1 (initial concentration of 1 μg/mL) was reduced by more than 98% by *A. auricular-judae*, 70% by *B. adusta*, 37% by *H. erinaceus*, and 50% by *L. edodes*, respectively, within 24 hr. The AFB_1 biodegradation was enhanced in the presence of NADPH and $NaIO_4$. No growth of the 5 AFB_1-degrading mushrooms was observed on cumarin agar plates and no decolorization of Remazol Brilliant Blue R agar plates was found from 4 mushrooms except *B. adusta*.

Significance: These results indicate that the extracts from the selected mushrooms, especially edible *Auricularia auricular-judae* and *Lentinula edodes*, which are major mushrooms produced commercially in Korea, have potentials for biodegradation of AFB_1 in food and feed.

Poster

P3-121 Effects of Various Antioxidants on Natural Spoilage Microflora, Lean Color and Sensory Characteristics of Retail Case-ready Top Sirloin Steaks

BRITTNEY BULLARD, Ifigenia Geornaras, Jennifer Martin, Dale Woerner, Robert Delmore and Keith Belk
Colorado State University, Fort Collins, CO

❖ ***Developing Scientist Competitor***

Introduction: Microbial growth, color degradation, off-odors and/or off-flavors associated with rancidity or spoilage contribute to the shelf-life of fresh beef.

Purpose: This study evaluated the effect of four antioxidants on the shelf-life of retail case-ready top sirloin steaks.

Methods: Beef top sirloins from a commercial beef plant were stored (4°C) in their original packaging for 15 days (to represent the average time wholesale meat products are stored before retailers process them) before portioning and application of antioxidant treatments. Each top sirloin steak was randomly assigned to a treatment (Control [no antioxidant], Antioxidant-A, Antioxidant-B, Antioxidant-C and Antioxidant-D). Antioxidant treatments were prepared and applied to steaks according to manufacturer instructions. Steaks were placed on foam trays, antioxidants sprayed, trays overwrapped, and then were packaged in low-oxygen mother bags, flushed with Tri-gas (modified atmosphere packaging; MAP) and stored (<4°C) for 6, 9, 12, 15, or 18 days. On each storage day, trays were removed from the mother bags and placed in retail display under florescent lighting. Microbiological (aerobic plate counts, lactic acid bacteria counts; *n*=5) and sensory analyses were performed at 0, 96, 120 and 144 h of retail display. Objective and subjective color were evaluated at 12-h intervals.

Results: At the end of retail display, Antioxidant-A- and Antioxidant-D-treated steaks were redder ($P<0.05$) than the control steaks. Antioxidant-D-treated steaks consistently had lower ($P<0.05$) spoilage bacterial populations compared to all other treatments. The percent beefy/brothy flavor of steaks decreased ($P<0.05$) as retail display hour increased for all antioxidant treatments. For all treatments, oxidized flavor of the steaks increased ($P<0.05$) as storage time in MAP or retail display increased. Antioxidant-A-, Antioxidant-C- and Antioxidant-D-treated steaks displayed lower ($P<0.05$) oxidized flavor compared to the control.

Significance: Overall, Antioxidant-A or Antioxidant-D improved the shelf-life of retail case-ready top sirloin steaks compared to the other tested treatments.

P3-122 Influence of Desiccation on Survival and Dry-heat Resistance of Long-term-survival Phase *Salmonella* Typhimurium and *Salmonella* PT 30 on Paper Discs and Raw Almonds

FEI WANG, Aubrey Mendonca, Aura Daraba, Yutong Zhang, David Manu, Angela Shaw and Byron Brehm-Stecher
Iowa State University, Ames, IA

❖ ***Developing Scientist Competitor***

Introduction:

During adverse environmental conditions in nature or food processing environments bacteria may remain in a long-term-survival (LTS) phase for months or years. In this phase, pathogens may become stress-hardened and resist antimicrobial interventions.

Purpose: Studies were performed to determine the influence of desiccation on survival and dry-heat resistance of LTS phase *Salmonella* Typhimurium and *Salmonella* PT 30 on paper discs and raw almonds.

Methods: LTS *Salmonella* cells were developed in tryptic soy broth with 0.6% yeast extract (TSBYE) at 35°C for 14 days. Exponential- and stationary-phase cells grown in TSBYE (35°C) for 4 h and 24 h, respectively, served as controls. Cells (10^7 CFU/mL) from each physiological state were desiccated on paper discs and raw almonds at 22 ± 1°C for 24 hours. Heat (100°C, 30 min) resistance of desiccated *Salmonella* on raw almonds was evaluated. Survivors were enumerated by plating diluted (10-fold) samples on tryptic soy agar with 0.6% yeast extract and counting bacterial colonies after incubation (35°C, 24 h).

Results: LTS *Salmonella* exhibited the highest desiccation and heat resistance ($P < 0.05$). Log reductions of exponential, stationary, and LTS cells on paper discs were 6.34, 4.39 and 2.46 for *S.* Typhimurium, and 6.5, 3.75, and 2.62 for *S*. PT30, respectively ($P < 0.05$). After desiccation on almonds, log reductions were 4.24 (exponential), 2.54 (stationary) and 1.97 (LTS) for *S.* Typhimurium; and 3.03, 2.58, and 1.76 for *S*. PT 30. Heating eliminated exponential phase survivors and decreased stationary and LTS cells, respectively, by ~1.82 and 1.58 log (*S*. Typhimurium) and ~2.28 and 1.85 log (*S*. PT30).

Significance: Results demonstrate that the LTS state cross-protects *S.* Typhimurium and *S*. PT30 against desiccation and heat, and should be considered when developing dry-heat processes for destruction of those pathogens on raw almonds.

P3-123 Growth of Fungi in Low Oxygen and Intermediate Water Activity Model Systems

ELIZABETH BUERMAN, Randy Worobo and Olga Padilla-Zakour
Cornell University, Ithaca, NY

❖ ***Developing Scientist Competitor***

Introduction: Hot-filled products are expected to have long shelf lives, however there are heat resistant, osmophilic fungi that can grow under extremely low oxygen conditions. Very few studies have been published regarding vacuum strength in low water activity (a_w), hot-filled products and the resulting oxygen concentration in the headspace nor the ability of known heat resistant spoilage fungi to grow at these levels.

Purpose: The objective of this study was to evaluate the effect of a_w, vacuum strength and oxygen concentration of hot-filled bottles and the survival of spoilage fungi *Byssochlamys, Talaromyces, Cladosporium, Penicillium* and *Aspergillus* under low oxygen conditions.

Methods: Glass bottles were hot-filled at 180, 190 and 200°F with sucrose, sodium chloride and water mixtures resulting in 0.70, 0.80, 0.90 and 1.00 a_w. Headspace oxygen and vacuum strength were measured using an AquaLab water activity meter and PreSens Fiber Optic oxygen meter, after equilibrium.

Spoilage fungi, isolated from foods, were inoculated into 70°Brix apple juice concentrate and Malt Extract Broth adjusted to 0.85, 0.90 and 0.93 a_w. Plates were incubated in anaerobic chambers adjusted to 400 ppb (1.0%) and 200 ppb (0.5%) oxygen for 60 days at 86°F.

Results: A linear trend in the hot-fill study was observed. Vacuum strength decreased and headspace oxygen increased as a_w decreased. At 0.70 a_w, the average vacuum was 8.3±0.3, 10.0±0.5, and 10.5±0.0 inHg and headspace oxygen concentration was 6.04±0.2, 5.5±0.1, 5.35±0.17 ppm at 180, 190 and 200°F respectively. At 1.00 a_w the average vacuum was 11.33±0.6, 12.7±0.3, and 13.7±0.6 inHg and headspace oxygen concentration was 5.42±0.2, 4.26±0.19, 3.82±0.37 ppm at 180, 190 and 200°F respectively. Spoilage fungi were capable of growth at reduced oxygen levels in low a_w models.

Significance: Our results will provide guidelines to control growth of heat resistant and osmophilic spoilage fungi in a_w controlled hot-filled foods.

Poster

P3-124 Detoxification of Aflatoxin B_1 by Cell-free Extract of *Aspergillus oryzae* MAO 103

KYU RI LEE, Sung Min Cho, Sung-Yong Hong and Soo-Hyun Chung
Korea University, Department of Integrated Biomedical and Life Science, Seoul, South Korea

❖ ***Developing Scientist Competitor***

Introduction: Aflatoxins are a group of mycotoxins which are produced mainly by toxigenic *Aspergillus flavus* and *Aspergillus parasiticus* and classified as a human carcinogen. Biological AFB_1-detoxification is the most desirable approach to reduce AFB_1 in foods.

Purpose: The aim of this study was to investigate the AFB_1-detoxification activity of cell-free extracts of *Aspergillus oryzae* MAO 103.

Methods: The AFB_1-biodegradation activities were investigated using cell-free extracts of *A. oryzae* MAO 103 (NCBI accession ID: KY020433). The AFB_1-detoxification activities of the extract were assayed under different conditions: pH (pH3~10), temperature (25~40°C), and cell cultivation time (8~16 days). The amount of AFB_1 was analyzed by HPLC-FLD.

Results: The cell-free extract of *A. oryzae* MAO 103 showed significant AFB_1-detoxification activities. AFB_1 was reduced by more than 97% within 1 day (initial concentration: 0.05 - 1 μg/mL of AFB_1) and about 90% of AFB_1 was decreased in 10 minute. The highest AFB_1-reduction was observed in the 14-day cell-free extract containing 823.2 μg/mL of total protein. The reaction was enhanced by NADPH (4 mmol) and $NaIO_4$ (1~3 mmol).

Significance: The cell-free extract of *A. oryzae* MAO 103 showed significant biodegradation activity toward AFB_1, indicating that the results could be applied to food and feed industries. The AFB_1-biodegradation activity by the cell-free extract of *A. oryzae* MAO 103 suggests the involvement of cytochrome P-450 monooxygenase in the detoxification reaction.

P3-125 Enumeration and Identification of Spoilage Lactic Acid Bacteria in Chilled Food Products Using 3M™ Petrifilm™ Lactic Acid Bacteria Count Plate

SITI AISHA ABD AZIZ[1], Su Ann Lee[1], Teen Teen Chin[1], Hui Key Lee[2], Chandraprasad S Rajangan[3] and Lay Ching Chai[2]
[1]ALS Technichem, Shah Alam, Malaysia, [2]University of Malaya, Kuala Lumpur, Malaysia, [3]3M Malaysia, Petaling Jaya, Malaysia

Introduction: Lactic acid bacteria (LAB) are the major bacteria groups associated to spoilage of food products, particularly in reduced oxygen packaging (ROP). Understanding the diversity of LAB associated to spoilage in ROP product and non-ROP product is essential in improving the product quality and shelf life that will lead to a reduction in food waste.

Purpose: Assess the impact of storage temperature on the abundance and diversity of LAB in selected ROP food products.

Methods: A total of 200 ROP chilled food products were purchased from the local retail. A portion of the samples were stored at 4°C or 25-27°C for 20h before the microbiological assessment was performed. Total aerobic bacteria (TPC) and LAB in food products were enumerated using 3M™ Petrifilm™ Aerobic Count Plate and 3M™ Petrifilm™ Lactic Acid Bacteria Count Plate, respectively. About 5-10 LAB colonies were isolated from each sample for bacterial identification.

Results: LAB were detected in 74% of the total samples, that ranged from 1.00-7.48 $\log_{10}$CFU/g, with significantly higher counts in ROP products including chicken sausage, chicken rolls and mushrooms (P<0.01) compared to produce, sauces and starchy foods. The LAB counts in food products stored at room temperature were significantly higher compared to before and after storage at 4°C (P<0.05). ROP products stored at 4°C for 20h did not significantly increase the total bacteria count (P>0.05), but the LAB had a significant increase (P<0.01) with about 1.02 $\log_{10}$CFU/g increment for LAB.

Significance: LAB were present in the majority of the chilled food products included in this study, particularly in ROP products. Refrigeration and ROP arrest only the growth of aerobic bacteria, but not LAB that may cause spoilage. Quantification and control of LAB are essential to optimize shelf life of food ROP products.

P3-126 Storage-Life and Microflora of Vacuum-packaged Pork Loin Cuts in Relation to Beef from the Same Abattoir

Mohamed K Youssef[1], Frances Tran[2], **PEIPEI ZHANG**[2], Colin O Gill[2] and Xianqin Yang[2]
[1]Cairo University, Giza, Egypt, [2]Agriculture and Agri-Food Canada, Lacombe, AB, Canada

❖ ***Developing Scientist Competitor***

Introduction: Pork or beef in chilled, vacuum packaged (VP) form is widely traded domestically and internationally. In recent years, substantial increase in the storage life of beef has been reported for Canadian and Australian products. In contrast, recent reports on the storage life of vacuum-packaged pork cuts are largely lacking.

Purpose: To determine the current shelf-life and microbiological conditions of chilled VP pork and beef.

Methods: Beef and pork sub-primal cuts obtained from a federally inspected Canadian research abattoir were vacuum-packaged on the day of production and stored at 2 and -1.5°C. Organoleptic assessment and microbiological analysis were performed in triplicates at day 0 and at appropriate intervals for up to 190 days of storage. Selected bacterial isolates were identified to species level by 16S rRNA gene sequencing.

Results: The odours of beef stored at 2°C for ≤ 110 days or -1.5°C for ≤ 190 days were mostly acceptable. Acid or dairy odours for VP pork stored at 2°C for ≤ 60 days or at -1.5°C ≤ 120 days were detected upon opening, but dissipated after a 2 h display. Before storage, the numbers of total aerobes were approximately 2 and 3 log CFU/cm² for pork and beef, respectively, with the beef microflora being mainly strict aerobes and pork microflora including a substantial fraction of species of *Enterobacteriaceae*. At the end of storage life, lactic acid bacteria were predominant in the microflora of beef at either temperature and pork at 2°C, while *Rahnella aquatilis* accounted for >50% of the final microflora on pork stored at -1.5°C .

Significance: The attainable storage life of Canadian VP pork and beef primal cuts is at least 120 and up to 190 days at -1.5°C. The microflora on beef and pork was different, indicating the animal sourced contamination.

P3-127 Variability in Growth Behavior of *Carnobacterium* Isolates in Medium with Low Initial pH

PEIPEI ZHANG and Xianqin Yang
Agriculture and Agri-Food Canada, Lacombe, AB, Canada

❖ ***Developing Scientist Competitor***

Introduction: *Carnobacterium* spp. are frequently isolated from vacuum-packaged (VP) beef, the pH of which is relatively low, around 5–6. Previous studies have demonstrated that the predominance of certain *Carnobacterium* strains in the final microflora may be linked to the storage life of VP beef.

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Information on the growth behavior of Carnobacteria would then be helpful to better understand the development of bacterial community structure on VP beef.

Purpose: To evaluate the growth behavior of *Carnobacterium* isolates at pH conditions relevant to VP beef.

Methods: The growth of 36 *Carnobacterium* isolates from VP meat cuts obtained from three abattoirs (A, B, and C) were determined in broth medium with initial pH 5.4 at 30°C via measurement of optical density at 600 nm (OD_{600}). F-test was performed to determine whether the growth kinetics, including detection time, growth rate, and ΔOD (the difference of OD_{600} between initial and stationary growth phase), were different among abattoirs, species, strains, and isolates. If $P < 0.05$, t-test was further used for pairwise comparison. The minimum pH (pHmin) allowing growth in BHI was further determined for each isolate.

Results: In medium with initial pH 5.4, detection time for strain NFU35 was significantly ($P < 0.05$) shorter than MMF-23, MFPB14D06-04, and G117. G117 showed the smallest ΔOD, followed by MFPB, NFU35, and MMF-23. The overall difference in growth kinetics among species was not significant, and the average detection time for isolates from abattoir A was longer than the other two abattoirs due to the presence of strain G117. The pHmin of G117 was 5, higher than the other isolates (4.4–4.8).

Significance: The different resistance of *Carnobacterium* strains to relatively low pH is likely one of the reasons resulting in difference in the microbial structure, which in turn lead to different storage lives of VP beef.

P3-128 Anti-listerial Activity of Lactic Acid Bacteria Isolated from Artisanal Cheeses Produced in the State of Minas Gerais (Brazil)

FERNANDA BOVO CAMPAGNOLLO[1], Larissa Pereira Margalho[1], Bruna Akie Kamimura[1], Verônica Ortiz Alvarenga[1], Vasco A. P. Cadavez[2], Ursula A. Gonzales-Barron[2] and Anderson Sant'ana[1]

[1]University of Campinas, Campinas, Brazil, [2]School of Agriculture, Polytechnic Institute of Braganza, Braganza, Portugal

Introduction: Brazilian artisanal cheeses, among them Minas cheeses, are highly appreciated by consumers. Nonetheless, some cheeses have been regularly found to harbour high-risk pathogens, such as *Listeria monocytogenes*, mainly arising from their elaboration with raw milk and informal production. However, cheese matrices are complex systems whose microbial competing factors (i.e., indigenous lactic acid bacteria (LAB)) and intrinsic properties (acidity, temperature, and water activity) can act as effective hurdles against *L. monocytogenes* proliferation.

Purpose: The aim of this study was to evaluate the capacity of LAB strains isolated from Minas artisanal cheeses as anti-listerial agents.

Methods: A total of 891 LAB strains were isolated from 244 Minas artisanal cheese samples (466 using MRS agar (MRSLAB) and 425 using M17 agar (M17LAB)), which were tested qualitatively for anti-listerial activity by the deferred antagonism assay at 30°C (24 h). Two *L. monocytogenes* strains, serotypes 1/2b and 4b, isolated from cheese and crude milk, respectively, were used. LAB strains with positive anti-listerial activity at 30°C were further tested at 7°C (10 days).

Results: MRSLAB strains presented significantly better anti-listerial activity at 30°C (73.0% and 70.8% for serotypes 1/2b and 4b, respectively) compared to M17LAB strains (21.2% and 23.1%, respectively), and there was no significant difference between listeria serotypes ($P<0.05$). Selected MRSLAB and M17LAB strains were able to keep anti-listerial activity at 7°C, with no significant differences between MRSLAB (96.8% and 97.2% for listeria 1/2b and 4b, respectively) and M17LAB (95.2% and 96.8%, respectively) or between *Listeria* serotypes ($P<0.05$).

Significance: These results suggested that LAB isolated from Minas cheeses using MRS agar present a better inhibitory effect against *L. monocytogenes* at different temperatures. These strains can be used as starter cultures in order to inhibit pathogen survival.

P3-129 Prevalence and Antibiotic Resistance of Bacteria Isolated from Retail Meats in Korea during the Year 2016

YONG HOON KIM

National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, Cheongju, South Korea

Introduction: Antimicrobials had been added to animal feeds for many years. Therefore, meticulous monitoring and surveillance to the antibiotic resistance of retail meats was necessary.

Purpose: The purpose of this study is to investigate prevalence and antimicrobial resistance of bacteria from retail meats in Korea during the year 2016.

Methods: We collected the retail meats in grocery stores periodically and conducted isolation of bacteria from the samples. With the isolates, we performed antimicrobial susceptibility testing using MIC, identification of antimicrobial resistance genes using PCR and MLST for the homology analysis.

Results: From the total 200 cases domestic food products, 88 *E. coli*, 34 S. aureus, 29 Enterococcus spp., and one *Salmonella* spp. were isolated. 200 imported samples were analyzed and 48 *E. coli*, 19 S. aureus and 3 Enterococcus spp. were isolated. *E. coli* from domestic primary products shoed higher resistance to tetracycline, nalidixic acid, ampicillin and streptomycin than the other antimicrobials, while for imported products streptomycin, tetracycline, ampicillin resistance was relatively higher. In case of S. aureus penicillin resistance was highest in both domestic products and imported products. In case of Enterococcus spp. resistances were rare except for daptomycin, tetracycline, quinuprostin/ dalfopristin. One *Salmonella* spp. showed resistance to only streptomycin. Two MRSA strains were isolated from domestic chicken meats. 6 ESBL producing *E. coli* were isolated from three domestic chicken meats and three imported chicken meats. By MLST analysis, two MRSA strains from chicken meats were determined as ST692 and that was similar to previous study in Korea. six ESBL ST types were ST23, 457, 602 from three domestic chicken meats and ST58, 117, 1286 from three imported chicken meats. Among the ESBL ST types, ST23, 457, 117 were isolated in previous years.

Significance: Our investigation indicates that retail meats are a possible source increasing the rates of antibiotic resistance in human. Therefore, continued monitoring and strengthened surveillance in relation to food safety, in particular to retail meats distributed to markets, are recommended.

P3-130 Antibacterial and Antioxidant Activity of Oregano Essential Oil on Stability of Low-acid Mayonnaise

IZABELA ALVES GOMES[1], Érika Fraga de Souza[2], Flávia dos Santos Gomes[2], Otniel Freitas-Silva[2] and Janine Passos Lima da Silva[2]

[1]Federal University of the State of Rio de Janeiro, Rio de Janeiro, Brazil, [2]Embrapa Food Technology, Rio de Janeiro, Brazil

Introduction: Low-acid mayonnaise produced with raw egg is a product rich in oil, almost a home-made product, but it is susceptible to lipid oxidation and microbial contamination by *Salmonella Enteritidis* (SE), which results in deterioration of the product and forms undesirable components such as free radicals.

Purpose: The purpose of this study was to evaluate the effect of using oregano essential oil (OEO) as a natural antibacterial and antioxidant in mayonnaise preparations with low-acidity.

Methods: The essential oil's antioxidant activity was evaluated using the DPPH assay. Samples were also evaluated for acidity, pH and water activity during 8 weeks. All experiments were performed in triplicate, expressing the results as averages of the three readings. The antimicrobial effect of OEO against SE was studied to be applied in mayonnaise during storage at 8° or 30°C for 24 hours. The data were submitted to analysis of variance (ANOVA) with 95% significance level (P <0.05) and the Tukey test (P < 0.05) using the statistical software XLSTAT version 7.5.

Results: The results showed that oregano essential oil is rich in phenolic compounds, with good antioxidant activity and acts to protect mayonnaise against oxidation reactions. There were statistically significant differences in the IC_{50} results (control sample 326 mg/mL; OEO sample 111 mg/mL). OEO also was very efficient against *Salmonella Enteritidis.*There were no colonies counted on the samples of mayonnaise with OEO, for control sample (without OEO), the results were 3.20; 3.10; 3.18 and 3.09 log CFU/g of mayonnaise for the times 0, 2, 4 and 24 hours respectively.

Significance: These results indicate that the use of OEO as a biopreservative (natural antimicrobial and antioxidant) can enhance food safety, serving as an additional barrier in helping the Good Manufacturing Practices and the Hazard Analysis Critical Control Point program, fundamental to food safety.

P3-131 Prevention of Mixed-species Biofilm Formations on Nanoscale Plasma-coated Surfaces

FNU CHENGGEER[1], Lin Li[1], John Jones[2], Meng Chen[2], Qingsong Yu[1] and Azlin Mustapha[1]

[1]University of Missouri, Columbia, MO, [2]Nanova, Inc., Columbia, MO

Introduction: Biofilm formation can lead to a series of important safety problems in the food industry. Low temperature plasma coating technology can inhibit initial cell adhesion and biofilm formation on surfaces by changing the surface energy and contact angle.

Purpose: To investigate the anti-biofilm activity of trimethylsilane (TMS) and TMS+oxygen (1:8) plasma coating on silicone and high density polyethylene (HDPE) food contact surfaces.

Methods: Silicone and HDPE wafers were coated with TMS and TMS+O_2 (1:8) plasma. Two groups of mixed-species biofilms, *Listeria monocytogenes* (LM) and *Salmonella enterica* (SE); and *Escherichia coli* O157:H7 (EC) and SE, were formed on the wafers for 48 h. The pour-plate method was conducted to determine bacterial counts.

Results: No significant reduction in counts of the mixed species biofilm of LM SCOTT A and SE I4-10 was observed on coated silicone. However, the same mixed groups were significantly reduced (>1 log CFU/wafer) on HDPE surfaces by the TMS+O_2 (1:8) treatment. Further, when comparing counts of LM in a single species biofilm with those in the mixed biofilm, the results showed a greater reduction in the mix which implied that antagonistic interactions among the LM and SE species were also responsible for its reduction. Counts of the mixed species biofilm of EC 505b with SE I4-10 on TMS+O_2 (1:8) coated silicone decreased by more than 1 log CFU/wafer. However, neither strain was affected by either plasma treatment on HDPE surfaces, indicating that the anti-biofilm efficacy of coated HDPE was less than that for silicone.

Significance: This study shows that TMS and TMS+O_2 (1:8)-coated silicone and HDPE surfaces could significantly inhibit certain mixed-species biofilms. Further, the interactions between the bacteria in a mixed biofilm also played a role in the reduction of a single species in the biofilm.

P3-132 Withdrawn

P3-133 Antimicrobial Activity of Fruit Extracts and Juice against Various *Listeria monocytogenes* and *Salmonella* Strains

Jingyi Du[1], Yi-Cheng Su[1], Christina DeWitt[1], Chengchu (Catherine) Liu[2] and **JOVANA KOVACEVIC**[3]

[1]Oregon State University, Astoria, OR, [2]University of Maryland Extension, Princess Anne, MD, [3]Oregon State University, Portland, OR

❖ ***Developing Scientist Competitor***

Introduction: Foodborne pathogens *Listeria monocytogenes* (Lm) and *Salmonella* spp. are especially problematic in ready-to-eat (RTE) foods. With consumer demands for healthy RTE food choices with minimal processing, there is a need for alternative methods to control these pathogens.

Purpose: This study investigated antimicrobial activity (AMA) of seven fruit extracts and juices against four Lm (Scott A, SFL0404, F5027, H0222) and four *Salmonella* strains (*S.* Weltevreden SFL0319, *S.* Newport ATCC 6962, *S.* Newport H1275, *S.* Typhimurium ATCC 14028) from human clinical samples, seafood, raw milk, produce, and meat sources.

Methods: Extracts (25-100% wt/vol) were prepared by blending fruit, or peel (1 min) and diluting with sterile distilled water. AMA was measured using well-diffusion assay on trypticase soy agar with 9 mm wells containing different concentrations of tested compounds (25-100%). For compounds that exhibited highest AMA, minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) were determined using tryptic soy broth (35°C, 48 h). Inoculum levels were ca. 6-7 log CFU/ml.

Results: Pomegranate peel extract (PPE) and cranberry juice (CJ) exhibited highest AMA, with average inhibition zones against Lm and *Salmonella* of 19.38 and 18.65 mm, respectively, for PPE, and 20.98 and 12.80 mm, respectively, for CJ. Limited inhibition (ca. 10 to 15 mm) was observed for blueberry, strawberry, plum meat, whole plum and pomegranate seed extracts. MBC of CJ against Lm and *Salmonella* was 25%, whereas MIC against Lm was 12.5%, but this concentration did not fully inhibit the growth of *Salmonella* strains. The MBC of PPE for tested strains was 37.5%, whereas the MIC of PPE against Lm and *Salmonella* were 13.5% and 22.5%, respectively.

Significance: This study demonstrates potential of PPE and CJ to be used as natural antimicrobials against Lm and *Salmonella* in RTE foods. However, more research is needed to optimize concentrations that exhibit effective AMA, but have minimal impact on sensory qualities of food products.

P3-134 The Synergistic Effect of the Photosensitizer Curcumin and Ascorbic Acid in Inactivating *Listeria monocytogenes* and *Escherichia coli* O157:H7

JINGWEN GAO[1], Junhua Han[2] and Karl Matthews[1]

[1]Rutgers University, New Brunswick, NJ, [2]Hebei University of Science and Technology, Shijiazhuang, China

Introduction: *Listeria monocytogenes* and *Escherichia coli* O157:H7 are foodborne pathogens linked to a great number of illnesses and economic losses, annually. Both pathogens have been isolated from fresh produce, meat and meat product, and poultry. Curcumin is the principle curcuminoid of turmeric and approved for food use in Europe (E100) and the USA. L-ascorbic acid (AA) is a GRAS substance commonly used as an antioxidant.

Purpose: The purpose of this study was to evaluate the synergistic effect of curcumin and AA in inactivating Gram positive and Gram negative foodborne pathogens.

Methods: Curcumin was dissolved in 95% EtOH and diluted to desired concentrations with sterile distill water (SDW). AA was dissolved in SDW and filter sterilized. Overnight cultures of *L. monocytogenes* L008 or *E. coli* O157:H7 86-24 were diluted to 6.0 log CFU/ml. Curcumin, AA, and cells were mixed in amber tubes and pre-incubated in the dark for 5 min. The mixture was then transferred to a 96-well plate and illuminated (450-455 nm, 0.13

W/cm^2) for 1 min (*L. monocytogenes*) or 10 min (*E. coli* O157:H7). Number of viable cells was determined; the detection limit was 2.4 log CFU/ml. Results were compared by ANOVA.

Results: A statistically significant, synergistic effect was observed for curcumin/AA concentrations ($P < 0.05$) in the inactivation of each pathogen. Based on direct plating, no viable *L. monocytogenes* were detected when exposed to 10 uM of curcumin and 250 ug/ml of AA; while *E. coli* O157:H7 were below detection limit following treated with 50 uM of curcumin and 25 ug/ml of AA. Populations of *E. coli* O157:H7 were 4.3 log CFU/ml and 5.9 log CFU/ml when exposed to 50 uM curcumin or 25 ug/ml AA, respectively.

Significance: The combination of photosensitizing curcumin and ascorbic acid has potential application as an antimicrobial treatment for inactivation of foodborne pathogens on the surface of meat, poultry, and fresh produce.

P3-135 Antimicrobial Effect of Copper-based Surfaces on Environmental Foodborne Pathogens Associated with Meat and Poultry Processing Facilities

ILAN ARVELO-YAGUA[1], Marcos X. Sanchez-Plata[1], Sergio Rocha[2], Patricia Landaida[2] and Luis Armijo[2]

[1]Texas Tech University, Lubbock, TX, [2]Agrosuper, Rancagua, Chile

Introduction: Foodborne pathogens can survive for long periods of time on environmental surfaces, and can become the source of contamination of ready-to-eat meat and poultry products. Feasible and practical alternatives for their control and elimination in high care areas is needed to reduce the risk of cross-contamination.

Purpose: Evaluate the viability and survival of *Salmonella* spp., and *Listeria monocytogenes* after inoculation on four different surfaces, including: two different copper-based alloys 1) C710, Cu 79% - Ni 21%, 2) C706, Cu 88.6% - Fe 1.4% - Ni 10%; and two common processing plant surfaces, 3) stainless steel (SS); and, 4) Teflon (TF).

Methods: Survival tests were conducted under controlled conditions at 8°C and 80% of RH. Surfaces were evaluated under clean and coated conditions (chicken fat). Over 230 coupons of 4 cm^2 were inoculated individually with a cocktail of each pathogen with a target concentration of 6.5 $\log_{10}$(CFU/cm^2) separately, and, the survivors were quantified at different time intervals (within 32 h) in order to determine the decimal time (*D*-value).

Results: No survivors were recovered from the dry and clean C706 surfaces (*D*-value of 0 h). The C710 eliminated *L. monocytogenes* instantaneously and had a *D*-value of 0.5 h for *Salmonella* spp. Clean and dry SS and TF had *D*-values higher than 8 h ($P < 0.05$). The presence of chicken fat increases the survival of the pathogens by protecting them from the contact with the antimicrobial surfaces and partial desiccation. However, in the presence of chicken fat, copper containing surfaces performed better that the clean SS and TF.

Significance: Copper containing alloy surfaces are effective at inactivating *Listeria monocytogenes* and *Salmonella* spp. under simulated commercial conditions and can potentially be used to control the persistence of foodborne pathogens in ready-to-eat environments.

P3-136 Cold Pasteurization of Tender Coconut Water by a Flow through Filter Column Containing Glass-beads Coated with Curcumin Nano-Liposomes

Jim Thomson[1], Nathalie Becerra-Mora[1], Punit Kohli[1], Derek Fisher[1], Victor Rodov[2] and **RUPLAL CHOUDHARY**[3]

[1]Southern Illinois University - Carbondale, carbondale, IL, [2]ARO, Rishon LeZion, Israel, [3]Southern Illinois University - Carbondale, carbondale, IL

Introduction: Tender coconut water (TCW) is one of most consumed natural energy drink. Although it is available in tropical countries, because of its natural health benefits it is increasingly consumed in other parts of the world. Therefore, there has been an increased requirement for packaging and shipping. TCW is highly susceptible to microbial contamination during extraction and packaging, thus requires pasteurization. Thermal pasteurization leads to loss of flavor and color of coconut water.

Purpose: The purpose of this study was to test the feasibility of using a nonthermal method, natural antimicrobial curcumin, for pasteurization of TCW.

Methods: A cold pasteurization method was developed by passing the TCW at 4°C through a glassbeads filter medium, coated with 50mM curcumin encapsulating nano-liposomes, leading to extended release of the natural antimicrobial curcumin into the coconut water. *Listeria monocytogenes* and *Escherichia coli W1485* were used as model organisms to test effectiveness of cold pasteurization. Nano-liposomes were prepared by the film rehydration method to encapsulate curcumin. A glass pipette was filled with the immobilized nano-liposomes on glass-beads to form the filter medium. The antimicrobial effect was tested by passing inoculated TCW with Gram positive *L. monocytogenes* and Gram negative *E. coli* W1485 through separate columns. The effect of the residence time of TCW in the column was observed by passing the inoculated TCW through the column multiple times.

Results: Reduction of *L. monocytogenes* and *E. coli* W1485, in pasteurized TCW, were observed to be reduced two log and one log, respectively, after five passes. Further experiments are suggested with higher residence time of TCW in filter medium to acheive the desired five log reduction.

Significance: Curcumin nano-liposomes are a potential antimicrobial that may be useful in the development of cold pasteurization technology for TCW. Large scale filter medium may be engineered for mass production of pasteurized TCW.

P3-137 Antibacterial Characteristics of Anthocyanins Extracted from Wild Blueberries against Foodborne Pathogens

Yujie Zhang[1], Caihong Wei[1], Xiaohong Sun[1], Yingjie Pan[1] and **VIVIAN CHI-HUA WU**[2]

[1]Shanghai Ocean University, Shanghai, China, [2]U.S. Department of Agriculture-ARS-WRRC, Albany, CA

Introduction: Wild blueberries have rich bioactive compounds, such as polyphenols, phenolics, and organic acids. Previous studies demonstrated the antibacterial activity of blueberries against the growth of pathogenic bacteria.

Purpose: The objective of this study was to evaluate the antibacterial characteristics and mechanisms of anthocyanins extracted from wild blueberries (*Vaccinium angustifolium*) against four foodborne pathogens.

Methods: Wild blueberries were extracted using the methanol extraction method. The fraction of anthocyanins was separated from blueberry extract by the C-18 column. *Listeria monocytogenes* (NO.12), *Staphyloccocus aureus* (AB91093), *Salmonella* Enteritidis (CMCC50041), and *Vibrio parahaemolyticus* (VP6) were used for investigating the antibacterial effects of anthocyanins. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of anthocyanins were determined by a twofold serial dilution method. The ability of anthocyanins to alter the membrane integrity of pathogens were visualized using scanning electron microscopy (SEM). After anthocyanins treatments, the metabolism of four pathogens was assessed by the value of OD_{630}. The total protein content, ATP enzyme, and AKP enzyme were measured with protein assay kits.

Results: The MBCs of anthocyanins against *L. monocytogenes*, *S. aureus*, *S.* Enteritidis, *V. parahaemolyticus* were 0.53 mg/mL, 0.27 mg/mL, 0.53 mg/mL, and 0.13 mg/mL, respectively. SEM confirmed that anthocyanins destroyed the cell membrane of the four bacteria. When foodborne pathogens were treated with anthocyanins for 2 h, total protein content decreased and the enzyme activity of AKP and ATP decreased by 54.3% and 69.7%, re-

spectively. With increasing concentration of anthocyanins, the value of OD_{630} distinctly decreased, indicating bacterial metabolism was decreased. This may affect the energy transfer of bacteria and inhibit bacterial growth and reproduction.

Significance: This study demonstrated that anthocyanins affected bacterial metabolism and reduced the bacterial cell activity through destroying the cell membrane and the growth condition of bacteria. Wild blueberry anthocyanins have potential applications in preventive medicine and food safety.

P3-138 The Effect of Sugar Substrates on the Efficacy of Bacteriocins to Inhibit *Listeria monocytogenes*

DANIELLE ROBOCON, Michael Gänzle and Lynn McMullen
University of Alberta, Edmonton, AB, Canada

❖ ***Developing Scientist Competitor***

Introduction: Evidence suggests that *Listeria monocytogenes* may develop resistance to bacteriocins via cells' sugar transport systems which can be influenced by the sugar substrate provided for metabolism.

Purpose: This study determined if *L. monocytogenes* developed resistance to carnocyclin A and leucocin A, grown in the presence of different carbohydrates.

Methods: *Carnobacterium maltaromaticum* UAL307 and *Leuconostoc gelidum* UAL187 were used to produce carnocyclin A and leucocin A, respectively. The bacteriocins were purified using hydrophobic interaction and reverse phase high performance liquid chromatography. Minimum inhibitory concentrations (MIC) were determined for five strains of *L. monocytogenes* in broth and on agar. To determine the impact of sugar substrate on development of resistance, 25°C growth curves of each strain were conducted in basal media with a defined sugar substrate (glucose, sucrose, fructose, mannose, cellobiose) with the addition of carnocyclin A or leucocin A.

Results: The concentration of leucocin A that inhibited the growth of *L. monocytogenes* was lower than the concentration of carnocyclin A; however, there were differences between liquid- and solid-phase systems. In broth, all strains grew in the presence of 2.2 mM leucocin A; however, on agar the MIC ranged from 11.7 to 62.5 µM leucocin A, depending on the strain. The MIC for carnocyclin A in broth was 0.83 mM to >6.7 mM, whereas on agar it ranged from 2.5 mM to >10 mM, depending on the strain. Leucocin A reduced growth rates most notably when strains were grown in glucose, fructose, and cellobiose. The lag phase was not affected by the sugar substrate. Carnocyclin A reduced growth rates among all strains compared to the control for each sugar; however, significant differences were noted among strains grown in cellobiose. The lag phase was increased for all strains in the presence of carnocylcin A, independent of sugar substrate.

Significance: This research indicated that sugar substrates influenced the sensitivity of *L. monocytogenes* to bacteriocins, which could allow for growth in foods.

P3-139 Reduction of Molds and *Listeria Monocytogenes* on Flour Tortilla Utilizing Targeted Directional Microwave Technology

KATHLEEN FERMIN[1], Don Stull[2], Andreas Neuber[1], Kendra Nightingale[1] and Mindy Brashears[1]
[1]Texas Tech University, Lubbock, TX, [2]MicroZap, Lubbock, TX

Introduction: Molds and *Listeria monocytogenes* are ubiquitous in nature. Molds are major spoilage agents especially in baked products, while *L. monocytogenes* is a foodborne pathogen capable of causing serious illness in susceptible populations.

Purpose: To determine the efficacy of targeted directional microwave (TDM) to reduce molds and *L. monocytogenes* on packaged flour tortillas.

Methods: In separate trials, flour tortillas were spot-inoculated with a two-strain mold and a five-strain *L. monocytogenes* cocktail at final inoculation level of 10^4 CFU/g. In duplicate, the samples packed in individual bags were subjected to five treatments using TDM with varying amounts of microwave energy, exposure time, and temperature change. Samples were serially diluted and plated onto dichloran rose-bengal chloramphenicol agar (for mold enumeration) and modified oxford agar (for *L. monocytogenes* enumeration) followed by incubation at 25°C for 4 days and 37°C for 24 hours, respectively. Non-inoculated tortillas without preservatives were also treated and set aside at room temperature to observe signs of mold growth.

Results: There were significant reductions ($P<0.05$) of both molds and *L. monocytogenes* on flour tortillas after treatments all but one TDM treatment. Four out of five treatments had mold reductions of 3.2 to 4.5 $\log_{10}$ CFU/g. A 2.0 to 3.9 $\log_{10}$ CFU/g reduction of *L. monocytogenes* on treated samples was observed except those from treatment with the lowest energy level. Non-inoculated preservative-free tortillas from two treatments with highest energy levels did not show signs of mold growth 30 days after production date, while all the untreated control samples had visible mold growth 5 days post-production.

Significance: Results suggest that TDM is an effective intervention to control molds and *L. monocytogenes* on flour tortillas, potentially improving shelf life and product microbial safety.

P3-140 Behavior of *Listeria monocytogenes* on Mortadella Formulated Using a Natural, Clean Label Antimicrobial during Extended Storage at 4° or 12°C

JOHN LUCHANSKY[1], Stephen Campano[2], Marcus Rieker[3], Laura Stahler[1], Lianna McGeary[1], Bradley Shoyer[1], Laura Shane[1], Manuela Osoria[1] and Anna Porto-Fett[1]
[1]U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, [2]Hawkins, Inc., Glen Allen, VA, [3]Rieker's Prime Meats, Philadelphia, PA

Introduction: Natural antimicrobials, such as buffered vinegar, have been used to control *Listeria monocytogenes*(Lm) in/on several ready-to-eat (RTE) meats. However, there is a scarcity of information published on the efficacy of natural antimicrobials used to enhance the safety of specialty/ethnic RTE meats.

Purpose: Validate efficacy of buffered vinegar (BV) or a blend of potassium lactate and sodium diacetate (KLac) to control Lm on mortadella, a specialty luncheon meat.

Methods: All-pork, mortadella was produced by a local butcher with or without 1.0 or 1.5% of liquid BV (LBV), 0.6 or 1.0% of dry BV (DBV), or 2.5% of KLac. In each of three trials, mortadella was sliced (ca. 1.5 cm thick, ca. 30 g) and surface inoculated with 250 µl per side of a five-strain mixture of Lm (ca. 3.8 log CFU/slice). The packages were vacuum-sealed and then stored at 4° or 12°C for up to 120 days.

Results: In the absence of antimicrobials, Lm numbers increased by ca. 2.3 and 5.7 log CFU/slice after 120 days at 4° and 12°C, respectively. With inclusion of 1.0 or 1.5% LBV, 1.0% DBV, or 2.5% KLac as ingredients, pathogen numbers decreased by ca. 0.4 to 1.0 log CFU/g after 120 days at 4°C, whereas inclusion of 0.6% DBV resulted in a ca. 0.2-log CFU/slice increase in Lm numbers. At 12°C, inclusion of 2.5% KLac, 1.0% LBV, or 0.6% DBV resulted in a ca. 1.3-, 1.7-, and 4.5-log increase in Lm numbers, respectively. However, when 1.5% LBV or 1.0% DBV were included in the formulation,

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pathogen numbers decreased by ca. 0.3 and 1.5 log CFU/slice, respectively, after 105 days, but product quality was lessened at this abusive storage temperature.

Significance: Inclusion of 1% LBV or DBV, as clean label ingredients, in mortadella is equally effective as 2.5%KLac (1.0 log reduction) to control Lm on mortadella during proper storage.

P3-141 Efficacy of Chlorine Dioxide Gas to Decontaminate Fresh Produce Used for In-store and Vendor Juicing Operations

MIKAYLA GOODMAN[1], Mark Berrang[2], Judy Harrison[1] and Mark Harrison[1]

[1]University of Georgia, Athens, GA, [2]U.S. Department of Agriculture-ARS-USNPRC, Athens, GA

❖ ***Developing Scientist Competitor***

Introduction: Unpasteurized juices are increasingly popular as consumers aim to find convenient, healthy menu options. Contamination of produce with pathogens such as *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* poses a risk. In-store treatment of small batches of mixed types of fresh produce with chlorine dioxide gas may prove effective in decontaminating produce before preparing juices.

Purpose: The purpose of this study was to determine the effectiveness of chlorine dioxide gas generated in self-contained sachets suitable for use in retail operations to reduce levels of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* in mixed produce containers.

Methods: A mixed cocktail of five strains of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* was spot inoculated on injured and uninjured oranges, kale, celery, and cucumbers. Produce was treated together in batches with 20 mg/kg ClO_2gas, 200 ppm chlorine, or 80 ppm peracetic acid; non-gas and water controls were also included. Two samples of each produce type from each treatment were subjected to microbiological analysis. Reductions of pathogens were compared through a random effects model with interactions.

Results: There were significant differences ($P<0.05$) between type of produce, microorganism, injury status, and treatment though treatment type proved to be the major source of variance. ClO_2 gas treatment on average for all produce types, microorganisms, and injury status produced a log reduction of 2.66 which was significantly different from all other treatments and controls. Peracetic acid and chlorine treatments were not found to be statistically different and produced 1.96 and 1.89 log reductions, respectively. The largest log reduction of 3.70 was with *E. coli*O157:H7 on injured kale that received gas treatment.

Significance: ClO_2 gas generated in self-contained sachets may be a practical, convenient delivery method and superior to commonly used sanitizers in decontaminating mixed types of produce used in retail juicing operations.

P3-142 Development of Antimicrobial Hydrogel Patches to Control *Listeria monocytogenes* in Foods Consumed Raw

HYEMIN OH, Sejeong Kim and Yohan Yoon

Sookmyung Women's University, Seoul, South Korea

❖ ***Developing Scientist Competitor***

Introduction: Since heat and antimicrobials cannot be applied to the foods consumed raw such as beef tartare and Sashimi, food safety for the foods has been issued. κ-carrageenans are linear polysaccharides sulfated galactan, and it has hydroxyl group in structures which could be useful in forming hydrogel.

Purpose: The objective of this study was to develop an antimicrobial hydrogel patch composed of κ-carrageenan to inhibit *L. monocytogenes* in foods consumed raw.

Methods: κ-carrageenan-based hydrogel was prepared by dissolving 1, 2, and 3% of κ-carrageenan powder in 25 ml distilled water, mixed with co-polymers (1% w/w; sodium alginate, xanthan gum, and collagen), and mixed with crosslinkers $CaCl_2$ and KCl. The mixture was heated at 98°C and stirred until a homogeneous solution was obtained. The κ-carrageenan-based solution was poured into plastic plate and dried at 15°C for 20 min. The obtained κ-carrageenan gels were cut into 1.0 × 1.0 cm, and polymer properties (strength, elasticity, swelling and antimicrobial loading capacity) were determined. The hydrogel patches were placed in 1 ml of 0.1-3.0% natural antimicrobials (grapefruit seed extract, citrus peel extract, and ε-polylysine) from 30 min to 4 h, and the hydrogel patches were used for a diffusion assay on tryptic soy agar plus yeast extract (TSAYE).

Results: Addition of KCl (0.2%) or $CaCl_2$ (0.2%) into κ-carrageenan increased the hydrogel strength, and the elasticity of the hydrogel gel was increased by adding collagen (1%) into κ-carrageenan. Among tested natural antimicrobials, 3% ε-polylysine was the most appropriate to inhibit *L. monocytogenes* growth. Thus, the antimicrobial hydrogel composed of 1% κ-carrageenan, 0.2% KCl, 1% collagen, and 3% ε-polylysine were prepared. The hydrogel patches formed clear zones (0.8 mm) of *L. monocytogenes* on TSAYE agar.

Significance: These results indicate that the developed hydrogel patch should be useful in controlling *L. monocytogenes* in food consumed raw.

P3-143 A Cranberry Extract as a Marinade Inhibits Growth of *Listeria* on Chicken

Archana Vasanthakumar, **CHAYAPA TECHATHUVANAN** and Margarita Gomez

Ocean Spray Cranberries, Inc., Lakeville-Middleboro, MA

Introduction: Cranberry extracts, natural, plant-based products, have been shown to inhibit foodborne pathogens as well as spoilage organisms *in vitro*. Our previous studies have also demonstrated microbial susceptibility to cranberry extracts in broth and liquid food systems.

Purpose: The goal of this study was to expand the applications of a cranberry extract as natural antimicrobial on a solid food system.

Methods: *L. innocua* was used as a surrogate for *L. monocytogenes*. Unprocessed chicken slices were inoculated with *L. innocua* and air-dried prior to exposure to cranberry-based marinades. Untreated but inoculated chicken, as well as water-treated chicken slices were included as controls. At 30 minutes and 3 hours post treatment under refrigeration, samples were enumerated for *L. innocua*. Each experiment was carried out in triplicate. Student's t-test was used for statistical analysis of the efficacy of microbial growth inhibition.

Results: Exposure to a marinade containing cranberry extract significantly resulted in a 2-log reduction ($P < 0.05$) of *L. innocua* on chicken slices with 3 hours post-exposure. A 30-minute exposure resulted in a 1-log reduction of *Listeria* on chicken.

Significance: Cranberry-derived products provide attractive alternatives to existing naturally occurring antimicrobials in food systems. The findings of this study showed that a cranberry extract could have widespread use to control *Listeria* sp. on unprocessed meat and poultry products.

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P3-144 Essential Oils in the Control of Planktonic and Sessile CELLS of *Staphylococcus Aureus*

TENILLE RIBEIRO DE SOUZA[1], Letícia Andrade do Vale[1], Kalmia Kniel[2] and Roberta Hilsdorf Piccoli[1]
[1]UFLA, Lavras, Brazil, [2]University of Delaware, Newark, DE

Introduction: *Staphylococcus aureus* is a Gram-positive pathogenic bacterium, present in food, capable of producing enterotoxins which may be involved in outbreaks of foodborne illness. *S. aureus*forms biofilms on biotic and abiotic surfaces. These biofilms may be a constant source of contamination; therefore, require proper management.

Purpose: The objective of this work was to evaluate the antimicrobial activity of *Cinnamomum cassia*, *Cinnamomum camphora* and *Litsea cubeba* essential oils (EO) on planktonic and sessile *Staphylococcus aureus*GL 5674 cells, as well as to evaluate the action of the combination of these essential oils on biofilms formed by these bacterial strains on AISI 304 stainless steel, with a skim milk substrate.

Methods: To evaluate the antimicrobial activity of the EO, the MBC (minimum bactericidal concentration) of the EO tested on planktonic cells was determined, as well as the MBC_B(biofilm minimum bactericidal concentration). In planktonic cells, the EO MBC was determined using the broth microdilution test in 96-well microdialysis plates. The final EO concentrations ranged from 0.078% to 10%. The bacterial culture was standardized in 10^8 CFU/mL. In sessile cells, biofilm was formed in microdialysis plate for 72 hrs at 37°C, and the EOs were tested in concentrations ranging from 0.078% to 10%, for 20 minutes. Sanitizing solutions based on the combinations of *C. cassia*, *C. camphora* e *L. cubeba*EOs were assessed on biofilms formed by GL 5674 strain in UHT skim milk after 72h of incubation.

Results: *C. cassia*, *C. camphora* e *L. cubeba* showed MBCs of 0.3125% ,1.25% and 0.63% respectively on GL 5674 planktonic cells. When in biofilm, this concentration was 4 times higher. The sanitizing solutions based on EOs were able to significantly reduce bacterial populations by 2.5 log CFU/cm^2 (P<0,05) in relation to the control.

Significance: The combination of these EO presents promising results as sanitizers for use in the food industry.

P3-145 Antimicrobial Ability of Modified Bacterial Cellulose Film

WEI WANG, Francois Xavier Nayigiziki, Polly Liou, Azlin Mustapha and Mengshi Lin
University of Missouri, Columbia, MO

Introduction: Bacterial cellulose (BC) is widely used in developing packaging materials. However, its poor solubility and flexibility can hinder its modification.

Purpose: The aim of this study was to improve the flexibility and antimicrobial ability of BC by mixing BC with Polyvinyl Alcohol (PVA) and incorporating nanoparticles into the film.

Methods: BC was synthesized by statically growing *Gluconobacter xylinus* in Hestrin and Schramm (HS) medium at 26°C for 7 days. Its flexibility was improved by mixing the BC slurry with 3% PVA solution. Nanosilver (AgNPs) was incorporated in the film by immersing the film in $AgNO_3$ solution and $NaBH_4$solution (R film) or simply mixing AgNPs with the film solution (M film). The two films were used in antimicrobial tests against various foodborne pathogens via an agar plate assay and by incubation in tryptic soy broth containing the films and plating at different time points over a 24-h period.

Results: The R film exhibited an obvious inhibition zone against *Escherichia coli* O157 in the agar assay. In contrast, no inhibition zone was observed for the control and M films. The number of *E. coli* O157:H7 grown with the R film was 22% lower than the other groups after 3 h incubation, 25% lower after 6 h and 15% lower after 24 h. This indicated that the R film in which the AgNPs were evenly dispersed has higher antimicrobial efficacy against *E. coli* O157:H7.

Significance: Development of antimicrobial packaging material using BC may provide a more efficient way to control foodborne pathogens.

P3-146 Effect of Acidified Peroxyacetic Acid on the Microbiological and Color Characteristics of Beef Tissue

BRIANNA BRITTON[1], Kathryn McCullough[1], Ifigenia Geornaras[1], Dale Woerner[1], Robert Delmore[1], Jennifer Martin[1], James Reagan[2] and Keith Belk[1]
[1]Colorado State University, Fort Collins, CO, [2]Zoetis, Parsippany, NJ

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Introduction: The beef industry continues to seek antimicrobial treatments that can effectively reduce bacterial contamination without affecting quality attributes of the final product.

Purpose: This study evaluated effects of acidified peroxyacetic acid (PAA) on *Escherichia coli* populations inoculated onto beef trimmings and head meat, and its effect on natural microflora and color of ground beef from treated trimmings.

Methods: In phase I, trimmings and head meat (n=5) were separately inoculated (6-7 log CFU/g) with a five-strain mixture of non-pathogenic *E. coli*. Samples were spray-treated (10-s) with PAA (345-360 ppm), a sulfuric acid+sodium sulfate blend (SSS; pH 1.0 or 1.2), PAA (345-360 ppm) acidified with SSS (aPAA; pH 1.0 or 1.2), lactic acid (LA; 4%), or water. Untreated (control) and treated samples were analyzed for Enterobacteriaceae (EB) counts. In phase II, uninoculated trimmings (n=5) were spray-treated with the same treatments, except water, and were then ground (with and without antioxidant addition), packaged under modified atmosphere (MAP), stored (0°C, 21 days), and then placed in simulated retail display (0-4°C, 5 days). Ground beef samples were analyzed for aerobic plate counts (APC), and objective and subjective color characteristics were obtained during retail display.

Results: The most effective (P<0.05) spray treatment against inoculated EB populations on trimmings was aPAA, with 0.6 log CFU/g lower counts than those of the control. Surviving inoculated populations on antimicrobially-treated head meat were not (P>0.05) different to those of water-treated samples. In phase II, LA- and aPAA-treated ground beef stored for 21 days under MAP conditions had APC that were 3.0 and 2.7 log CFU/g lower (P<0.05), respectively, than those of control samples. Trained panelists scored aPAA-treated ground beef most similar (P>0.05) in redness and discoloration to that of the control through day-3 of retail display.

Significance: With appropriate application, aPAA can be an effective alternative to LA for improved shelf-life of ground beef.

P3-147 Antimicrobial Activity and Mechanism of Garlic (*Allium sativum*) Extracts against *Aeromonas hydrophila*

YOU JIN KIM, In Young Choi, Won Keun Song, Dong Wook Jang, Hae-Yeong Lee, Min-Jeong Lee and Mi-Kyung Park
Kyungpook National University, Daegu, South Korea

Introduction: Garlic (*Allium sativum*) belongs to the Lilliaceae family and has a wide range of antimicrobial activity against bacteria, fungi, protozoa, and viruses. Although many studies have been focused on antimicrobial activity of garlic extracts, they have not been focused on *Aeromonas* strains.

Poster

Purpose: The purpose of this study was to investigate the antimicrobial activity and antimicrobial mechanisms of garlic extracts against *A. hydrophila*.

Methods: Garlic was extracted with water and ethanol at 22°C and 90°C, respectively. The antimicrobial activity and MIC test of each extract were investigated using the disc diffusion method. The impact of garlic extracts on cell wall synthesis of *A. hydrophila* was examined using a TEM. Bradford assay was performed to examine the protein leakage from the cell wall and inhibition of protein synthesis.

Results: Water and ethanol garlic extracts at 22°C showed greater antimicrobial activities against all 9 *Aeromonas* strains than water and ethanol garlic extracts at 90°C. The ethanol garlic extract at 22°C showed the highest antimicrobial activity against *A. hydrophila* SNUFPC A7 with a clear zone size of 18.33±0.58 mm. An MIC test confirmed that water and ethanol garlic extract at 22°C exhibited the most potent antimicrobial activities against *A. hydrophila* JUNAH, SNUFPC A3, SNUFPC A7, SNUFPC A8, SNUFPC A9 at 10 mg/disc. TEM images of *A. hydrophila* SNUFPC A7 after exposure to garlic extract showed noticeable changes in the cell membranes of *A. hydrophila* presumably due to the outflow of cell constituents. Bradford assay confirmed that both water and ethanol extract damaged the cell membranes of *A. hydrophila* SNUFPC A7. The leakage of bacterial protein increased significantly as incubation time was increased.

Significance: This study demonstrated that water and ethanol garlic extract at 22°C showed antimicrobial activity against *Aeromonas* strains by changing its cell walls and affecting protein synthesis.

P3-148 Evaluation of *Psoraleae semen* Extract as a Natural Antimicrobial Agent for Food Application

SEJEONG KIM[1], Yohan Yoon[1] and Kyoung-Hee Choi[2]

[1]Sookmyung Women's University, Seoul, South Korea, [2]Wonkwang University, Iksan, South Korea

Introduction: Natural substances are spotlighted as promising alternative antimicrobial agents because they are relatively low toxic to human cells and exert few influence on environment. *Psoraleae semen,* the seed of natural herb *Psoralea corylifolia L.*, has been used as a traditional Asian medicine.

Purpose: In this study, *Psoraleae semen* was evaluated in *in vitro*and in sausage for its application as a natural antimicrobial agent in food and pharmaceutical fields.

Methods: Antibacterial effects of 69 therapeutic herbal plants extracts were investigated on 9 bacterial strains through a disc diffusion assay. Among them, the antimicrobial activity of *Psoraleae semen* was further evaluated using MIC (minimal inhibition concentration) and biofilm formation assays. The effect of *Psoraleae semen* extract on bacterial cell membranes was determined by measuring the leakage of 280 nm-absorbing materials and observing the bacterial cell through scanning electron microscope (SEM) in the presence of the extract. Finally, the antimicrobial activity of *Psoraleae semen*was confirmed in sausages.

Results: Disc diffusion assay determined that the extract displayed no antimicrobial effect on Gram-negative bacteria, but significant effect on Gram-positive bacteria. The MICs of *Psoraleae semen* extract were determined as 8 µg/mL for *Streptococcus mutans*, and 16 µg/mL for *Enterococcus sp.* and *Staphylococcus aureus*. Furthermore, biofilm formation was inhibited at 8-16 µg/mL of the extract. Measurement of 280 nm-absorbing materials leakage and SEM images revealed that cell membranes of Gram-positive bacteria were damaged by the exposure to the extract during bacterial growth. The growth of *Listeria monocytogenes* in sausage was hindered during storage in the presence of the extract at 4°C for 10 days.

Significance: These results showed that *Psoraleae semen*extract could be applied for the growth control of Gram-positive bacteria as a natural antimicrobial agent in food and pharmaceutical industries.

P3-149 Antioxidant Activity and Influence of Extracts of *Citrus* Byproducts on Adherence and Invasion of *Campylobacter jejuni*, as Well as on the Relative Expression of *Cad*f and *Cia*b

Norma Heredia[1], **JORGE DÁVILA-AVIÑA**[2], Sandra Castillo[2] and Santos Garcia[1]

[1]Universidad Autonoma de Nuevo Leon, San Nicolas, Mexico, [2]Universidad A. de Nuevo Leon, San Nicolas, Mexico

Introduction: *Campylobacter jejuni* is one of the most important foodborne pathogens in the world. Adherence and invasion are key processes during the development of infection of *Campylobacter jejuni*. Considering the increasing incidence of antibiotic resistance, it is crucial that new strategies be developed to control this pathogen.

Purpose: In this study, the use of three citrus byproducts, *Citrus limon*, *Citrus aurantium*, and *Citrus medica*, to prevent adherence and invasion of *C. jejuni* in HeLa cells was evaluated. The effects of these extracts on expression levels of genes involved in adherence and invasion processes (*cadF* and *ciaB*) were also examined. Furthermore, were determined the antioxidant activities and the contents of phenolics and flavonoids in these extracts.

Methods: Extracts were added to cultures of *C. jejuni* and determined the adherence/invasion ratio to HeLa cells. The relative expression of *cad*F and *cia*B, and the housekeeping gene *glyA* was determined by real time qRT-PCR. Total contents of phenolic and flavonoid compounds, DPPH radical scavenging activity, and the Trolox-equivalent antioxidant capacity of extracts were determined by spectrophotometric methods.

Results: In general, byproduct extracts at subinhibitory concentrations affected adherence (reduced 2.3 to 99%) and invasion (reduced 71.3 to 99.2%) to HeLa cells. The expression of *cadF* and *ciaB*, was reduced 66 to 99% and 81 to 99% respectively, depending on strain or extract added. The total phenolic content of byproducts varied from 92 to 26 mg GAE/g, and total flavonoids from 161 to 29.29 mg QE/g. *C. aurantium* showed the highest % of radical scavenging activity (RSA, 90.1).

Significance: *Citrus* byproduct extracts could be good alternatives for devising new strategies to control *Campylobacter* infections.

P3-150 Gaseous Ozone and Low-temperature Treatment for Controlling Growth of *Aspergillus parasiticus* in Peanuts

JING NI TAN[1], Cheng-An Hwang[2] and Hsin-I Hsiao[3]

[1]National Taiwan Ocean University, Keelung, Taiwan, [2]U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, [3]National Taiwan Ocean University, Keelung, Taiwan (ROC)

Introduction: Peanuts are susceptible to contamination of mold spores. During peanut storage, the growth of molds such as *Aspergillus parasiticus*on peanuts may produce mycotoxins and could cause adverse health effects to human when consumed.

Purpose: The objective of this study was to determine the effects of combining gaseous ozone and storage temperature for decontaminating and preventing the growth of *A. parasiticus*in peanuts.

Methods: Peanuts were inoculated with spores of *A. parasiticus* and exposed to ozone gases of 0 (control), 40, 60, 80, and 100 µmol for 60, 120, and 180 mins. Treated peanuts were stored at 5, 18, and 25°C, and *A. parasiticus*counts and thiobarbituric acid reactive substances (TBARS, a lipid oxidation indicator) were determined during storage.

Results: The initial level of inoculated *A. parasiticus* in peanuts was approximately 8 log CFU/g. After ozone treatments, a decrease of >7 log CFU/g of *A. parasiticus* was observed for treatments with 100 µmol ozone for ≥60 mins. During storage, mold growth on these treated peanuts did not occur at 5 and 18 °C. The effective ozone treatments for reducing *A. parasiticus* on peanuts and preventing its growth during storage were 100 µmol ozone with a 60-min exposure time and storage temperature of ≤18°C. These treatments also did not cause significant lipid oxidation in peanuts as indicated by the TBARS levels during storage.

Significance: The results indicated that ozone treatment combining with low temperature storage could effectively eliminate *A. parasiticus* contamination and growth on peanuts. The method may be incorporated into peanut processing to reduce the contamination and growth of molds on peanuts.

P3-151 Inactivation of Bacteria Using Single Wavelength 405 nm Light

STEPHEN LYON

Sealed Air Corporation, Sturtevant, WI

Introduction: Vital Vio LLC of Troy, NY has developed a system containing light emitting diodes that give off a single wavelength of light at 405 nm. This is a visible form of light that is near the border between visible and ultraviolet light. This wavelength of light is capable of producing "reactive oxygen species" (ROS) within bacteria that leads to cell death.

Purpose: This study tested the efficacy of bacterial inactivation over time using a continuous 405 nm light source.

Methods: Glass slides were coated with equal volumes of a bacterial suspension (*Staphylococcus aureus* or *Pseudomonas aeruginosa*). The dried slides were placed on shelves that were two and eight feet from the light source. On the opposite side of the experimental set up there were two shelves at the same height intervals that contained an equal number of bacteria-coated glass slides, but the light source was from fluorescent fixtures on the ceiling of the laboratory (controls). Triplicate samples from each shelf (treatment and controls) were collected at times: 0, 0.5, 1, 2, 4, 7, 14, 21, 28, and 35 days for *Staphylococcus aureus* and 0-28 days for the *Pseudomonas aeruginosa.*The concentration of live bacteria on each slide was determined by classical methods of serial dilution and plating onto a standard growth medium.

Results: There was a small (0.5-2 log) but statistically significant (95% confidence interval) difference between the bacteria exposed to the 405 nm light and the ambient light for the *Staphylococcus aureus,* while *Pseudomonas aeruginosa* analyses yielded mixed results with regards to statistically significant differences.

Significance: The combination of continuous exposure to low-level biocidal light plus episodic cleaning/sanitizing of food-related surfaces can improve pathogen control.

P3-152 Antibiotic Resistance of Beneficial Lactic Acid Bacteria Isolated from Smoked Salmon

LUIS NERO[1], Bernadette DGM Franco[2], Elisabetta Tome[3] and Svetoslav Todorov[4]

[1]Universidade Federal de Viçosa, Vicosa, Brazil, [2]University of São Paulo, São Paulo, Brazil, [3]Universidad Central de Venezuela, Caracas, Venezuela (Bolivarian Republic of), [4]Universidade Federal de Viçosa, Viçosa, Brazil

Introduction: *Lactobacillus* spp., *Pediococcus* spp. and *Enterococcus* spp. are wide spread lactic acid bacteria (LAB) in fermented foods. Their potential as probiotic brings an interesting opportunity to explore them as tools for novel functional food products. However, before using beneficial strains in foods, it is necessary to verify their safety including their resistance to antimicrobials, a current concern in public health.

Purpose: This study aimed to investigate the antimicrobial resistance profile of LAB strains isolated from smoked salmon, previously characterized as potential probiotics.

Methods: *Lb. curvatus* ET06, ET30 and ET31, *Lb. fermentum* ET35, *Lb. delbrueckii* ET32, *P. acidilactici* ET34 and *E. faecium*ET05, ET12 and ET88 were isolated from smoked salmon, identified based on their biochemical and genetic characteristics (including 16s rRNA sequencing), and characterized as possessing probiotic characteristics. The strains were subjected to PCR to verify the presence of 23 genes related to antibiotic resistance, and also to resistance/sensitivity to 31 antibiotics.

Results: All tested LAB were sensitive to amikacin, metronidasole, oxacillin and vancomycin. Strains ET05, ET12, ET31, ET35 and ET34 were resistant to ciprofloxacin; ET05 to furazolidone; ET05, ET12, ET30, ET31, ET35, ET32 and ET34 to kanamycin; ET06 to nitrofurantion; ET05, ET31, ET32 and ET34 to tobramycin; ET05, ET30, ET31, ET35, ET32 and ET34 to trimethoprim. All tested LAB contained antibiotic resistance genes: 5 genes in ET06 and ET35; 6 genes in ET30, ET05 and ET34; 8 genes in ET31 and 9 genes in ET12, ET88 and ET32.

Significance: Despite the beneficial properties of the tested LAB, presence of antibiotic resistance genes are of concern as horizontal gene transfer to pathogenic bacteria hampers their application as probiotics cultures. Acknowledgments: CNPq, CAPES, FAPESP, FAPEMIG

P3-153 Development of Antimicrobial Susceptibility amongst Microbiota from Hospices in South Africa

JANE NKHEBENYANE[1], Ryk Lues[2] and Teboho Mokoatsi[2]

[1]Central University of Technology, FS SA, Bloemfontein, South Africa, [2]Central University of Technology, FS, Bloemfontein, South Africa

Introduction: High prevalence of resistant and multi-resistant bacterial strains worldwide is placing a significant burden on healthcare settings and the society at large. Environmental contamination in hospices may contribute to transmission of healthcare pathogens when food handlers contaminate their hands. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major public health concern and is responsible for both hospital and community-associated infections worldwide. Moreover, *Acinetobacter baumannii* is a gram-negative opportunistic bacterium that can cause infectious outbreaks in critically-ill patients, often with limited treatment options due to antibiotic resistance

Purpose: The purpose of this study was to compare antimicrobial susceptibility profile among microbial flora isolated from foodhandlers' hands and food preparation surfaces in 15 hospice kitchens around Central South Africa.

Methods: Minimum inhibitory concentration was determined on 100 strains using the agar dilution method according to the CLSI. Antibiotic powders obtained from Sigma were as follows: chloramphenicol, gentamicin, oxacillin, cefoxitin, nalidixic acid and tetracycline. The inoculum was prepared by direct suspension of colonies from overnight cultures (Mueller-Hinton agar), into 9ml saline solution to achieve a suspension equivalent to 0.5 McFarland standards. The plates containing doubling antibiotic concentrations (0.25–256 $mg.ml^{-1}$) were inoculated with 1×10^5CFU/spot using a multipoint inoculator. MICs were read after 48h of incubation at 35°C.

Results: All the *Acinetobacter baumanii* strains were resistant to cefoxitin (0% susceptible). About 60% of *Staphylococcus aureus* isolates isolated from food handlers' hands were resistant to tetracycline (MIC range, 32-128 $mg.ml^{-1}$). *Enterococcus faecalis* strains were also resistant (90%) to gentamicin (MIC range, 0.25->128 $mg.ml^{-1}$).

Significance: The implication of data is that microorganisms from the hospice environment are becoming multidrug resistant compared to the previous years and this is of great concern to this vulnerable health care setting

Poster

P3-154 Applying N-Halamine Compound to Absorbent Pad for Controlling Spoilage-related Microorganisms in Refrigerated Beef

TIAN REN[1], Mingyu Qiao[1], Tung-Shi Huang[1], Jean Weese[1] and Yung-Hsiang Tsai[2]
[1]*Auburn University, Auburn, AL,* [2]*National Kaohsiung Marine University, Kaohsiung City, Taiwan*

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Introduction: Meat exudates immobilized in absorbent pads generate undesirable odors, spoil the food, and may promote the growth of foodborne pathogens. N-halamine compound, 1-chloro-2,2,5,5-tetramethyl-4-imidazoidinone (MC), has been reported as a promising antimicrobial agent with long-lasting antimicrobial activity. Incorporating N-halamine into the absorbent pads may reduce the microbial loads, increasing the quality of food products.

Purpose: The purpose of this study was to evaluate the antimicrobial effects of MC-treated absorbent pads in beef during the refrigerated storage.

Methods: Beef was packed with treated absorbent pads, preserved aerobically under refrigeration (4 ± 0.2°C) for 11 days. Microbial loads in absorbent pads and meat samples were analyzed on day 0 (three hours after packing), 1, 4, 7 and 11, by serial dilutions and plating on selective medial agars. Differences between treatments were analyzed with ANOVA Tukey test.

Results: Cellulose materials treated with MC reduced the levels of total aerobic bacteria, *Pseudomonas* spp., and lactic acid bacteria in absorbent pads to under the detection limit in the first days. During the rest days of storage, the microbial loads were statistically significant reduced ($P < 0.05$) in all MC treated absorbent pads. Meanwhile, microbial loads in meat samples also lowered remarkably on day 11 in the MC treated groups. The levels of *Enterobacteriaceae*from the treated samples remained under the detection limit during the entire storage period.

Significance: MC treated absorbent pads were able to reduce microbial loads in refrigerated beef, and thus have the potential to extend the shelf life of food products.

P3-155 Carbon Dots' Light-activated Antimicrobial Activities against Bacterial Pathogens

Mohamad Al Awak[1], Ping Wang[2], **XIULI DONG**[1], Yongan Tang[1], Ya-Ping Sun[2] and Liju Yang[1]
[1]*North Carolina Central University, Durham, NC,* [2]*Clemson University, Clemson, SC*

Introduction: Infectious diseases caused by bacterial pathogens have been a constant threat to the public health. Photo-activated antimicrobial technology is developing rapidly in response to the demand for development of effective treatments to control and prevent bacterial infections. Carbon dots (CDots) are generally small carbon nanoparticles with various surface passivation. The photoinduced redox processes in CDots have been credited for the photocatalytic activities that make carbon dots excellent candidates as antibacterial agents.

Purpose: The objectives of this study were to validate and compare the light-activated antibacterial function of CDots against Gram positive and Gram negative bacteria, to investigate the correlation of CDots' antibacterial function with its quantum yield, and to explore the optimization of CDots' antimicrobial activity by a combination of its properties and treatment conditions.

Methods: A series of specifically prepared Cdots with 2,2'-(ethylenedioxy)bis(ethylamine) as the surface functionalization molecule (EDA-CDots), whose fluorescence quantum yields (Φ_F) ranged from 7.5% to 27%, were synthesized. The traditional surface plating method was used to determine the viable cell reduction of *Escherichia coli* and *Bacillus subtilis* cells after treatment with these CDots under light illumination to evaluate the light-activated antibacterial function.

Results: Cdots' light-activated antibacterial function toward both *E. coli* and *B. subtilis,* under different light conditions, was validated and compared. Gram positive *B. subtilis* cells were more susceptible to CDots treatment. The results of this first study revealed there was a correlation between CDots' light-activated antibacterial efficiency and their quantum yields. A combination of the selected fluorescence quantum yields (Φ_F), dots concentration, and treatment time could achieve ~5 log viable cell reduction.

Significance: This study demonstrated that CDots have a great potential to be a class of low cost, low to no toxicity, highly efficient photo-activated antimicrobial agents against bacterial pathogens.

P3-156 Antimicrobial Activity of Kefir against *Cronobacter sakazakii* and Its Application

DONG-HYEON KIM[1], Dana Jeong[1], Il-Byeong Kang[1], Kwang-Young Song[1], Hong-Seok Kim[1], Young-Ji Kim[1], Hyunsook Kim[2] and Kun-Ho Seo[1]
[1]*Konkuk University, Seoul, South Korea,* [2]*Hanyang University, Seoul, South Korea*

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Introduction: *Cronobacter sakazakii* is a life-threatening foodborne pathogen found in powdered infant formula and dairy products. Kefir is a dairy probitotic product and its antimicrobial activity against various foodborne pathogens including *C. sakazakii* was reported in our previous study.

Purpose: On the basis of these results, we applied the antimicrobial activity of kefir supernatant to controlling *Cronobacter sakazakii* in powdered infant formula (PIF). In addition, we tested the antimicrobial activity of culture supernatants derived from individual lactic acid bacteria in kefir to identify key microorganisms that mediate these effects.

Methods: The spot on lawn method and growth curve analysis were employed for the screening of the antimicrobial activity of kefir against *C. sakazakii*. In addition, individual kefir isolates were isolated and tested for their antimicrobial activity. To address the mechanisms, their metabolites were analyzed by using HPLC.

Results: We found no viable *C. sakazakii* cells remaining in PIF rehydrated with 30% kefir supernatant solution for 1 h, demonstrating the antimicrobial activity of kefir supernatant against *C. sakazakii* could be applied in real food samples. In addition, a total of 20 *C. sakazakii*strains—including 10 clinical and 10 food isolates—was completely inhibited in the presence of kefir supernatant. We found that *L. kefiri* exerted strongest antimicrobial effects against *C. sakazakii* among all isolates although the supernatant of *L. kefiri* has higher pH and lower titrable acidity. Considering that the organic acid spectrum and pH neutralization reduced the *L. kefiri*-dependent growth suppression, it is inferred that this activity is mainly due to synergistic effect of various organic acids produced by heterofermentation of the strain.

Significance: Our results highlight the applicability of kefir and its individual isolates for preventing *C. sakazakii* contamination in the food industry.

P3-157 Isolation and Characterization of a Novel Antimicrobial Exopolysaccharide Produced by *Lactobacillus* Kefiranofaciens DN1 from Kefir

DANA JEONG[1], Dong-Hyeon Kim[1], Il-Byeong Kang[1], Hyunsook Kim[2], Kwang-Young Song[1], Hong-Seok Kim[1] and Kun-Ho Seo[1]
[1]Konkuk University, Seoul, South Korea, [2]Hanyang University, Seoul, South Korea

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Introduction: Kefir is fermented milk produced by co-cultures of lactic and acetic acid bacteria and yeast that are capsulated by exopolysaccharide (EPS) matrix. Although our previous studies showed that kefir possesses antimicrobial effects against various foodborne pathogens, no EPS molecules contributing these effects have not been reported yet.

Purpose: The aims of this study were to isolate the EPS-producing lactic acid bacteria (LABs) from kefir, (ii) purify their EPS, (iii) and identify its antimicrobial activity against two major foodborne pathogens.

Methods: A total of 22 strains of LAB were isolated from kefir and the ability of each strain to produce EPS was determined by the ethanol and trichloroacetic acid precipitation method. In addition, the antimicrobial activities of the EPS produced by *Lactobacillus kefiranofaciens* DN1 (EPS_DN1) against *Listeria monocytogenes* and *Salmonella* Enteritidis were assessed by growth curve analysis. Finally, the monosaccharide composition of EPS_DN1 was determined by High-Performance Size-Exclusion Chromatography (HPSEC).

Results: A total of 22 strains, including eight strains of *L. kefiranofaciens*, two strains of *Lactobacillus kefiri,* seven strains of *Lactococcus lactis*, and five strains of *Leuconostoc mesenteroides*, were isolated from kefir. The ability of each strain to produce EPS was determined and *L. kefiranofaciens*DN1 was found to have the highest EPS yield among all isolates (2.20 g/L). Moreover, EPS_DN1 at a concentration of at least 1% was able to exert bactericidal effects against both pathogens. Interestingly, results of HPSEC analysis indicated that EPS_DN1 was not the major EPS of kefir called kefiran, suggesting that EPS_DN1 represents a novel bioactive compound.

Significance: EPS_DN1 is a novel polysaccharide produced by *L. kefiranofaciens* isolated from kefir, and could be applied to develop natural antibiotic alternatives.

P3-158 Decay Kinetics of Residual Peracetic Acid and Hydrogen Peroxide on Poultry and Red Meat

RICHARD WALSH[1], John Hilgren[1], Deborah Klein[2] and Oriana Leishman[1]
[1]Ecolab Inc., St. Paul, MN, [2]Ecolab Inc., Greensboro, NC

Introduction: The increase in the maximum allowable level of peracetic acid (PAA) in poultry and red meat process water to 2000 ppm PAA (poultry) and 1800 ppm PAA (red meat) has greatly expanded the use of this chemistry across the industry, adding to the safe consumption of these foods. In addition to broad-spectrum antimicrobial efficacy, one of the main benefits of peracids is the post-treatment breakdown to the innocuous components of acetic acid and water.

Purpose: To date, a study documenting the breakdown rate of peracetic acid and hydrogen peroxide following a dip treatment in PAA has not been conducted. The study presented seeks to provide documentation and determination estimates for zero-point residue levels for each of the chemical species peracetic acid and hydrogen peroxide.

Methods: Chicken and beef samples were dipped in commercially-available PAA slightly above the maximum allowable level of 2,000 ppm PAA for 30s as a worst-case treatment scenario. Samples were drained on a rack. Recovery and measurement of remaining PAA/Peroxide were determined by iodometric titration at time points.

Results: Peracetic acid level on chicken was below the limit of detection (<LOD) level at 31 minutes, with a regression fit of 0.97, and hydrogen peroxide level <LOD after 19 minutes, with a regression fit of 0.94. Peracetic acid levels on beef was <LOD after 4.2 minutes drain time, with a regression fit of 0.987, and hydrogen peroxide level <LOD after 14.3 minutes, with a regression fit of 0.99.

Significance: Decay kinetics of both the PAA and peroxide on the tissue demonstrated exponential decay, which bolstered support for the safe use of peracetic acid at levels up to 2,000 ppm in the processing of poultry or red meat. This data supports the use of PAA by demonstrating that there are no or neglible residuals expected at time of consumption.

P3-159 Four Quaternary Ammonium-based Disinfectants Show Limited Efficacy for Inactivation of Human Norovirus GII.4 Sydney

JEREMY FAIRCLOTH, Emma Lepri and Lee-Ann Jaykus
North Carolina State University, Raleigh, NC

Introduction: Human norovirus (HuNoV) is a highly infectious foodborne pathogen that is difficult to inactivate even upon exposure to sanitizers and disinfectants applied under recommended use conditions. Quaternary ammonium compounds (QACs) are one such disinfectant category, but there is limited evidence of their efficacy against HuNoV.

Purpose: This study was undertaken to characterize the efficacy of four commercial QACs against HuNoV GII.4 Sydney and a cultivable surrogate, Tulane virus (TuV).

Methods: HuNoV GII.4 Sydney positive stool (20% suspension), TuV cell culture lysate, and GII.4 virus-like particles (VLPs) were used in this study. Virucidal suspension assays were conducted in accordance with ASTM International standard E1052-11 using exposure times of 5, 10, 20, and 30 min. Analysis of degree and mode of GII.4 HuNoV inactivation was evaluated using a combination of RT-qPCR with RNase pre-treatment and SDS-PAGE. Analysis of TuV infectivity was done by plaque assay using LLC-MK2 cells.

Results: RT-qPCR assay results showed a 1.1±0.2 and 1.8±0.2 $\log_{10}$ reduction in HuNoV genome copies after 10 and 30 min exposure to QAC4, respectively. QACs 1 through 3 produced <1 $\log_{10}$ reduction following exposures of up to 30 min. QAC4 was, therefore, chosen for additional screening with TuV and GII.4 VLPs. A 1.3±0.2 $\log_{10}$ reduction in TuV infectivity following a 0.5 min exposure to QAC4 was observed and infectivity was eliminated following a contact time of 5 min. SDS-PAGE of HuNoV VLPs exposed to QAC4 for up to 30 min showed minimal capsid damage, suggesting the mechanism of action is not peptide bond cleavage.

Significance: Collectively, this study demonstrated limited efficacy of QACs against HuNoV, but greater sensitivity of the TuV surrogate. The behavior of cultivable surrogates may not always be representative of HuNoV. Consistent with currently held beliefs, the QACs evaluated here, with currently available methods, would have only partial utility for HuNoV inactivation.

P3-160 Screening Food Microbiota for Novel Antimicrobial Compounds Suitable for Food Preservation

WALAA HUSSEIN[1] and Ahmed Yousef[2]

[1]Ohio State University, Columbus, OH, [2]The Ohio State University, Columbus, OH

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Introduction: The excessive use of antimicrobials has led to the decrease of their effectiveness against foodborne pathogens and spoilage bacteria. Therefore, innovations are needed for discovery of new potent antimicrobials to be used in food.

Purpose: The study was carried out to search for a novel, potent antimicrobial agent, purify it, and determine its physico-chemical characteristics.

Methods: Twenty samples of various fermented food products were screened for beneficial microorganisms that showed potent antimicrobial activity. Identification of the most potent bacterial strain was accomplished using16S rRNA gene sequencing. The antimicrobial compound was purified by cationic exchange resin and C_{18}silica cartridges. The antimicrobial compound purity and approximate molecular weight were determined by running Polyacrylamide gel electrophoresis (PAGE) and the stability at different temperatures, pH values, and treatment with different enzymes was tested.

Results: Out of 1500 tested isolate, an *Enterococcus durans* OSY-EGY strain, isolated from an Egyptian cheese, was found to produce an antimicrobial compound at pH 4.6, at a concentration of 800 arbitrary units/ml. This compound was the most active against *Listeria* spp., *Lactobacillus* spp., *Pediococcus* spp., and *Lactococcus lactis*. The antimicrobial compound retained its activity after storage at 4°C for 12 months, heating at 100°C for 80 minutes and 120°C for 25 minutes, treatment with alpha-chymotrypsin, trypsin, carboxypeptidase, aminopeptidase, papain enzymes, and over a pH range from 1.0 to 13.0. The antimicrobial compound was purified by cationic exchange resin and C_{18} silica cartridges with elution by dimethyl sulfoxide (DMSO), which indicated the compound's cationic and amphipathic nature. PAGE showed the antimicrobial compound as a single band with molecular weight of approximately 5200 kDa.

Significance: The investigated antimicrobial compound showed unique physico-chemical characteristics and stability to digestive enzymes, heat, and acidic and alkaline pH. These traits make it a good candidate as a food preservative.

P3-161 Plasmid Mediated Colistin Resistance in Food Animal Intestinal Contents Detected by Selective Enrichment

KIMBERLY COOK[1], Richard Meinersmann[2], Scott Ladely[1], Jodie Plumblee[1], Rachel Whitaker[1], Kay Williams[1], Uday Dessai[3] and Eileen Thacker[1]

[1]U.S. Department of Agriculture–ARS, Athens, GA, [2]U.S. Department of Agriculture-ARS-USNPRC, Athens, GA, [3]U.S. Department of Agriculture-FSIS, Washington, DC

Introduction: Colistin (polymyxin E) is a cationic polypeptide antibiotic that has broad-spectrum activity against Gram negative bacteria. It is classified as critically important in human medicine for treating hard-to-treat multi-drug resistant infections. Recently, a plasmid-mediated colistin resistance gene *(mcr-1)* was discovered in China followed by global reports of *mcr-1* genes in bacterial isolates from humans and livestock sources; however, information on prevalence of *mcr-1* in the U.S. was lacking. The presence of colistin resistance on a plasmid greatly increases the likelihood that *mcr-1* will be horizontally transferred among a broad spectrum of bacteria, including pathogens. Dissemination of *mcr-1* can potentially limit the lifespan of this critically important antibiotic.

Purpose: This study was conducted to determine the prevalence of plasmid-mediated colistin resistance in members of *Enterobacteriaceae* associated with U.S. food animal production.

Methods: Cecal (intestinal content) samples (*n*=2003) from swine, turkey, chicken, and beef cattle were selectively enriched in buffered peptone water with colistin (2 ug/mL) with overnight incubations and plated onto MacConkey's agar with colistin (2 ug/mL). Isolates were screened by PCR for the presence of the *mcr-1* gene.

Results: Two *Escherichia coli* isolates from two samples of swine intestinal contents were positive for the *mcr-1* gene, putting the overall prevalence of plasmid mediated colistin resistance in food animals at slaughter in the U.S. at 0.1%. One *E. coli* isolate was resistant to four other drugs (ampicillin, streptomycin, sulfisoxazole, and tetracycline) and the second isolate was only resistant to colistin. Both the strains of *E. coli* with the *mcr-1* gene are not commonly associated with human disease.

Significance: This study demonstrated that plasmid-mediated colistin resistance is rare in animals at slaughter in the U.S. and suggested that the potential for widespread dissemination of the *mcr-1* gene in this country, via food animal production, may be limited.

P3-162 In Vitro Evaluation of Essential Oils and Plant Extracts as an Alternative to Antibiotic Used in Pork Meat Production

Carmen M. S. Ambrosio[1], Natalia Y. Ykeda[1], Severino M. Alencar[1], Carmen J. C. Castillo[1], Andrea M. Moreno[2] and **EDUARDO M. DA GLORIA**[3]

[1]Universidade de São Paulo, Piracicaba - SP, Brazil, [2]Universidade de Sao Paulo, Sao Paulo, Brazil, [3]Universidade de Sao Paulo, Piracicaba - SP, Brazil

Introduction: The overuse of antibiotics in animal production is a reason for the emergence of bacterial resistance to antibiotics and the possibility of resistant bacteria or resistance genes transfer to the human food chain by pork products has been considered an indirect risk to public health. There is great concern about the emergence of plasmid-mediated colistin resistance (*mcr-1*) in *Escherichia coli*, a mechanism of resistance to polymyxins (colistin).

Purpose: This study evaluated the antibacterial activity of some essential oils (EOs) and plant extracts on four *Escherichia coli* isolates, which were isolated from pig gut.

Methods: Initial screening of antibacterial activity of eight citrus EOs (by-products of orange juice production), two propolis EOs, and extracts (ethanolic, hexanic, and isopropilic) from jalapeño (*Capsicum annum*) and bacupari (*Garcinia gardneriana*) was performed by disk diffusion method. Colistin was used as positive control.

Results: Three citrus EOs exhibited antibacterial activity (Brazilian orange terpens (BOT), Orange oil phase essence, and Tahiti lime oil phase) on *E. coli* isolates; analysis of variance (ANOVA) showed a significant difference for the antibacterial activity among them. Tukey's test ($p \leq 0.05$) and Principal Component Analysis (PCA) showed that BOT demonstrateded the largest antibacterial activity; showing superior action compared to colistin. Moreover, the results showed a significantly different sensitivity of isolates to citrus EOs, with isolate *E. coli* U21 (K88 LT/STb/F18/STa) being the most resistant. BOT was tested to determine its MIC using survival curves, resazurin test by microdilution method, and MBC (by plaiting) with *E. coli* U21. The MIC and MBC of BOT were 3.7 mg/mL. The CG/MS analysis of BOT showed that Limone, cis-limonene oxide, and myrcene were the major compounds.

Significance: EOs that are by-products of orange juice production could be an alternative control for *E. coli*. However, its use as alternative to synthetic antibiotics should be better verified by in vivo evaluations.

Poster

P3-163 Effect of Pimenta Malagueta *(Capsicum frutescens)* and Red Pepper *(Capsicum annuum)* Extracts on LuxRI-Type Quorum Sensing-regulated Phenotypes

MILAGROS LISETH CASTILLO RIVERA, Beatriz Ximena Valencia Quecan, Neuza Mariko Aymoto Hassimotto and Uelinton Manoel Pinto
Universidade de São Paulo, São Paulo, Brazil

❖ ***Developing Scientist Competitor***

Introduction: Quorum sensing (QS) inhibition leads to bacterial virulence interference without the use of antibiotics. Plants have been studied as sources of QS inhibitors, but there is a lack of appropriate controls in many published studies. The effect of compounds from *Capsicum* species on QS is unknown.

Purpose: This study aimed to evaluate the effect of two *Capsicum* species extracts on phenotypes regulated by LuxRI-type QS.

Methods: Extracts were obtained by solid phase extraction and compounds were quantified by High Performance Liquid Chromatography (HPLC). Methanol and methanol-amonium extracts were obtained for each pepper. Minimum inhibitory concentrations and growth curves were determined to test for cell growth inhibition. Violacein production was quantified in *Chromobacterium violaceum* ATCC12472. Biofilm formation and swarming motility were tested in *Pseudomonas aeruginosa* PAO1, *Serratia liquefaciens* MG1 and *Serratia marcescens* UFOP1.

Results: HPLC revealed luteolin and capsaicin as characteristic compounds in extracts. Growth inhibition was observed at concentrations greater than to 1.25 mg/ml. No significant differences (P>0.05) in violacein production were observed at sub-inhibitory concentrations (P>0.05). Biofilm formation was increased (P<0.05) in *S. liquefaciens* and it was unaltered on *P. aeruginosa*. Extracts did not inhibit swarming motility on *S. marcescens*, although colony morphology was different. *Serratia liquefaciens* motility was inhibited by methanolic extracts at 1.25 mg/ml, while *P. aeruginosa* motility was unaltered.

Significance: Our findings suggested that *C. frutescens* and *C. annuum* extracts do not specifically interfere with phenotypes regulated by QS. This was an important finding as it showed the lack of compounds in these plants that target QS in the selected bacteria. The use of appropriated controls and extract concentrations assisted in eliminating false positive QS inhibition.

P3-164 Antimicrobial Efficacy of *Syzygium antisepticum* Plant Extract against *Staphylococcus aureus* and Methicillin-resistant *S. aureus* and Its Application Potential with Cooked Chicken

Wenqian Yuan[1], **HYUN GYUN YUK**[2] and Hyun-Jung Chung[3]
[1]National University of Singapore, Singapore, Singapore, [2]Korea National University of Transportation, Chungju, South Korea, [3]Inha University, Incheon, South Korea

Introduction: Medicinal plants have been used for disease treatment for centuries, and many have recently been reported to possess good antimicrobial properties. However, thr majority of them remain unexplored

Purpose: This study aimed at identifying a novel plant extract with potential antimicrobial activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA), to validate efficacy in a food model, and to elucidate the major volatile composition and antimicrobial mechanism.

Methods: The minimum inhibitory and bactericidal concentrations of four plant extracts (*Atuna racemosa* AR, *Xanthostemon verticillatus* XV, *Syzygium antisepticum* leaf SA-L, and *Syzygium antisepticum* stem SA-S) were determined against *S. aureus* and MRSA, using the microdilution method. Anti-staphylococcal activity of SA-L was validated in cooked chicken at 4, 10, and 25°C. SA-L extract volatile composition was determined using GC-MS. The antimicrobial mechanism of the crude extract and its major compound was studied using transmission electron microscopy and flow cytometry, respectively.

Results: Among the four extracts evaluated, SA-L showed the most potent antimicrobial activity against *S. aureus* and MRSA, with an MIC of 0.13 mg/mL. In a food study, higher SA-L concentration (32 mg/mL) was needed to inhibit or reduce growth of *S. aureus* and MRSA in cooked chicken, but this caused a color change on meat surface. By GC-MS, caryophyllene (9.19%) was found to be the most abundant volatile compound in SA-L extract. Both crude extract and caryophyllene induced membrane damage in *S. aureus. C*aryophyllene, alone, showed weaker antimicrobial activities.

Significance: These results demonstrated the antimicrobial properties of *S. antisepticum* leaf extract against *S. aureus* and MRSA, identified its major volatile composition, and its membrane-damaging antimicrobial mechanism. However, further work is needed to enhance its antimicrobial activity in food matrices.

P3-165 Inhibition of Bacterial Quorum Sensing (QS) by Organic Extracts of Onion Varieties

BEATRIZ XIMENA VALENCIA QUECAN, Milagros Liseth Castillo Rivera, Neuza Mariko Aymoto Hassimotto and Uelinton Manoel Pinto
Universidade de São Paulo, São Paulo, Brazil

Introduction: Phenotypes controlled by quorum sensing (QS) include virulence factors that can influence food quality and safety. There is great potential presented by plant compounds to inhibit QS and its controlled phenotypes.

Purpose: The purpose of this study was to evaluate the quorum quenching potential of organic extracts obtained from red and white onion varieties against QS microbial models.

Methods: Phenolic compounds of red and white onions were extracted with organic solvents, then separated by solid-phase chromatography and identified by using HPLC and *LC-ESI-MS/MS.* The antimicrobial activity of the extracts was evaluated by determining the minimum inhibitory concentration (MIC) using broth microdilution method, testing concentrations between 125 to 15 µg/mL and by determining growth curves. The inhibitory effect on the QS system was tested, evaluating phenotypes regulated by the QS system such as violacein production in *Chromobacterium violaceum* ATCC 12472 and swarming motility in *Serratia liquefaciens* MG1 and *Pseudomonas aeruginosa* PA01.

Results: Quercetin 3,4'-diglucoside, Quercetin-3-glycoside and Isorhamnetin were the predominant compounds in white and red onion. Additionally, cyanidin-3-glycoside was found in red onion. The MICs for both types of onion were 125-62.5 µg/mL respectively. Concentrations below the MIC were used for the quorum quenching experiments. Violacein production by *C. violaceum* showed a reducing trend with red onion extract at a concentration of 31.2 µg/mL. Motility of *S. liquefaciens* showed a reduction in a concentration of 62.5µg/mL with red onion extract, while for *P.aeruginosa,* inhibition of motility was not observed.

Significance: The obtained results could boost new applications of onion varieties in the food industry, as future inhibitors of foodborne virulence factors. It is likely that the observed effects are due to the phenolic compounds that were identified here. Future studies will test the mechanism of inhibition presented by the identified compounds in the extracts.

P3-166 Independent Matrix Validations for the Detection of *Salmonella enterica* in 375 Gram Samples across Various Product Categories by the Atlas® *Salmonella* Sen Detection Assay

WILLIAM CHANEY[1], Benjamin Bastin[2], Patrick Bird[2], Joe Benzinger[2] and Erin Dreyling[3]
[1]*Roka Bioscience, San Diego, CA,* [2]*Q Laboratories, Inc., Cincinnati, OH,* [3]*Roka Bioscience, Warren, NJ*

Introduction: Diagnostic performance and accuracy of rapid pathogen detection methods may be adversely impacted by matrix type or other analytical parameters. Rapid method adoption and implementation should require validation for matrices pertinent to the stakeholder.

Purpose: A matrix validation was conducted for the Atlas® *Salmonella* SEN Detection Assay across various analytical parameters and food categories.

Methods: Eight matrices, each representing a category of finished product, were selected for matrix validation with the SEN assay. Each study consisted of 30, 375g *Salmonella* inoculated portions, of which 20 targeted fractional levels (0.2-2 CFU/portion) and 10 were inoculated to approach POD = 1 (2-10 CFU/portion). Inoculation serovars were stressed according to product type. After a stabilization period, sample replicates were enriched with BPW for 24-28 hours at 42°C. SEN assay results, which consisted of two analytical volumes at both 24 and 28 hours, where compared to results confirmed by the appropriate paired USDA MLG or FDA BAM reference culture method.

Results: For each matrix (low moisture cheese, hot dogs, tomato, liquid whole egg, peanut butter cream pie, buttermilk biscuit, caramel popcorn, and sunflower seeds) fractional positive results were obtained in the low inoculated replicates. For high level replicates, all were presumptive positive, except tomatoes, where 8 of 10 were presumptive. All presumptive results, at 24 and 28 hours for both 40 and 400 uL analytical volumes, aligned with culture results; the exception being 1 false positive in tomato and popcorn for a single assay across replicate iterations. There were no significant differences, by paired POD analysis, for the SEN assay as compared to the cultural results.

Significance: These data support the application of the SEN assay for the accurate analyses of matrices within the specified food categories and types evaluated.

P3-167 Validation of Individual and Wet Pooled Environmental Sample Analyses in Buffered Peptone Water and Lactose Broth by the Atlas *Salmonella* Sen Detection Assay

WILLIAM CHANEY[1], Benjamin Bastin[2], Patrick Bird[2], Joe Benzinger[2], Ali DeShields[3] and Erin Dreyling[4]
[1]*Roka Bioscience, San Diego, CA,* [2]*Q Laboratories, Inc., Cincinnati, OH,* [3]*Q Labs, Cincinnatti, OH,* [4]*Roka Bioscience, Warren, NJ*

Introduction: Wet compositing or "pooling" of analytical samples post-enrichment for rapid pathogen detection is a cost-effective and common practice, however, many methods have not been validated for this practice, which may increase false negative risk via dilution and loss of analytical sensitivity.

Purpose: To validate the Atlas *Salmonella* SEN Detection Assay for the analyses of individual or 5 sample wet composited environmental sponge samples enriched in BPW or Lactose broth.

Methods: Sixty, 4"×4" stainless steel coupons were utilized for surface inoculation. Ten replicates were non-inoculated, 40 were inoculated at a low-level and 10 at a high level to approach POD=1. Samples were inoculated with *Salmonella* Derby (ATCC #6960) and 10X *Citrobacter freundii*(ATCC #6879) and allowed to dry overnight. Surfaces were sampled with sponge swabs pre-hydrated with D/E neutralizing broth. Half of the test samples were enriched with 190mL BPW for 20 hours at 42°C and half with 225mL Lactose broth for 24 hours at 35°C. Post enrichment, 1ml of each sample was combined with 4mL of non-inoculated sample enrichment to form a 5 sample wet composite. Both individual and pooled samples were assayed by the SEN method in duplicate using 12 and 400µL analytical volumes as well as by FDA BAM 5 cultural analysis.

Results: For individual and wet pooled samples in BPW, SEN detected 9 and 5 presumptive positives from the low and high level replicates, respectively, and for the 12µL and 400µL analytical volumes. For individual and wet pooled samples in Lactose broth, SEN detected 8 and 5 presumptive positives across the study parameters. All presumptive results correlated 100% with FDA BAM 5 reference culture results.

Significance: These data support the application of the SEN assay for the accurate analyses of individual or 5 sample wet composited environmental samples enriched in BPW or Lactose broth.

P3-168 Development and Validation of a Novel, Enzyme-based Sample Preparation Step as a Workflow Modification for the Atlas® *Listeria* Environmental Detection Assay to Mitigate Free Nucleic Acid Detection Originating in Phage-based Processing Aids

WILLIAM CHANEY[1], Brett Maroni[1], Tucker Lopez[1], Kelly Cassutt[2], Celina Puente[1], Sarah Verver[1] and Christopher Haney[1]
[1]*Roka Bioscience, San Diego, CA,* [2]*Roka Bioscience, Warren, NJ*

Introduction: Bacteriophage based processing aids for pathogen reduction on food or in food processing environments contain residual analyte that can cause false positive results when using rapid pathogen detection methods.

Purpose: This study was conducted to develop and validate a sample preparation solution for mitigating environmental free nucleic acid detection using the Atlas®*Listeria* Environmental Detection Assay.

Methods: A phage based processing aid was serially diluted and assayed with the Atlas® *Listeria* spp. Detection Assay to estimate load of residual analyte in the product. An enzyme treatment to degrade free RNA was investigated and application parameters (concentration, temperature, time, and environment) optimized to evaluate direct and indirect treatment of post-enriched environmental samples by the Atlas® *Listeria* Environmental Detection Assay in the presence of the processing aid. To validate the optimized solution, 60 environmental swabs were collected from a facility, 30 of which were used to swab 4" x 4" coupons treated with the processing aid and another 30 without treatment for inoculation with *Listeria* spp. All swabs were enriched and assayed by the Atlas®method, with and without the novel sample preparation, and by cultural analysis.

Results: The processing aid product, alone, contained greater than 10^{10}CFU equivalents of free, residual analyte. Enzyme efficacy at 50U/reaction was markedly improved when samples were prepared with a wash step and optimized reconstitution buffer as compared to direct treatment of enrichment sample. For validation with Atlas®, all 30 uninoculated samples from processing aid treated surfaces were falsely positive by standard assay and 29 were resolved when assayed with the novel sample preparation treatment. From inoculated samples, 22 culture confirmed samples were detected by both the standard and modified assays.

Significance: The described sample preparation solution effectively mitigates free nucleic acid detection by the Atlas® LE Detection Assay while providing an additional tool for troubleshooting environmental positive samples.

P3-169 Development of a Bead-based Flow Cytometry Immunoassay for the Simultaneous Detection of Foodborne Bacterial Pathogens in Poultry and Meat Products

CELIA SUAREZ PANTALEON[1], Alexandre Monteforte[1], Benoit Granier[1], Delphine Larose[2], Cindy Dropet[2], Nicolas Desroche[2], Patrice Arbault[2], Anne-Catherine Huet[3], Riccardo Marega[3], Philippe Delahaut[3] and Nathalie Gillard[3]
[1]Unisensor SA, Ougree (Liege), Belgium, [2]NEXIDIA SAS, Dijon, France, [3]CER Groupe, Health Department, Marloie, Belgium

Introduction: Rapid and multiple detection of foodborne pathogens is a key element to guarantee safe food and prevent foodborne illness. Shiga-toxin producing *Escherichia coli* (STEC) and *Salmonella spp.* are amongst the most prevalent pathogens found in meat, poultry and derived products, thus representing an important human health threat.

Purpose: The purpose of this work was to develop a bead suspension Flow Cytometry Immunoassay for the simultaneous detection of *E. coli* serotypes O157:H7 and O26, and 5 serovars of *Salmonella enterica enterica* (*S.* Enteritidis, *S.* Typhimurium, *S.* Virchow, *S.* Newport and *S.* Meleagridis).

Methods: Polyclonal antibodies were produced against specific strains of *E. coli* O157:H7 and *E. coli* O26, whereas a generic polyclonal antibody was generated to cover the 5 *Salmonella* serovars. Individual bead sandwich assays were developed, and then combined in a triplex bead- test to simultaneously detect *E. coli* O157:H7, *E. coli* O26, and the 5 O-groups of *Salmonella*.

Results: The multiplex assay presented a Limit of Detection in buffer of 10^4-10^5 CFU mL^{-1}. No additional cross-reactivity was found with other serotypes of *E. coli*, *Salmonella* serovars, or additional Enterobacteria, with the exception of the test O157:H7 which showed positive signal with *S*. Urbana [shared antigen O:30 (N)], and the test *Salmonella* which recognized *S*. Montevideo [shared antigen O:7 (C1) with *S*. Virchow]. Different enrichment broths were evaluated to determine the recovery of foodborne pathogens in artificially contaminated meat samples (beef and poultry). Matrix interference, influence of competitive flora, and sensitivity for all the media have been compared. Two broths have shown higher growth rates and better recoveries.

Significance: These results indicate that bead-based Flow Cytometry Immunoassays are suitable for the simultaneous, rapid (less than two hours) and high-throughput detection of pathogenic Enterobacteria in food commodities. Further work is ongoing to expand the scope of the test to other significant *E. coli* serotypes and *Salmonella spp.*

P3-170 Evaluation of Growth Kinetics of Diverse *Salmonella* in Modified Bam Preenrichment for Shell Eggs

MELANIE BUTLER, Anna Laasri, Thomas Hammack and Gina Ryan
U.S. Food and Drug Administration, College Park, MD

Introduction: The FDA BAM method for detection of *Salmonella* in shell eggs uses a more concentrated preenrichment medium (1:2 egg:TSB) than 1:9 sample:broth ratio found in the BAM for other foods. Evidence shows strain differences and contamination levels may reduce outgrowth levels in 24-hour preenrichment cultures. The sensitivity of the current culture method permits detection of a single organism through multiple enrichment steps; however typical qPCR assays require higher cell concentrations (above 4-log). Factors affecting qPCR sensitivity and performance that may lead to false negative results include differences in genetic diversity (e.g., divergent alleles in target sequence), strain biology and cell injury incurred from prior exposure to specific egg factors (e.g., yolk or albumen).

Purpose: The purpose of this study was to evaluate factors affecting *Salmonella* growth dynamics in concentrated shell egg preenrichment, such as cell history, genetically diverse strains, and contamination ratio.

Methods: Homogenized albumen and whole egg aliquots were contaminated with eight egg and clinical *Salmonella* strains, representing seven serotypes, at two concentration levels. Preenrichment samples were inoculated with cold-adapted samples per BAM protocol. *Salmonella* growth was determined by plate count methods. Growth parameters were analyzed by the Baranyi model.

Results: Overall, growth rates in concentrated BAM shell egg preenrichment were similar for all strains evaluated. Lag phase duration was significantly different ($P < 0.001$) for serotype, contamination level, and prior exposure to albumen or whole egg at low temperature. However, maximum growth in preenrichment cultures exceeded 7-log cell concentration for all samples tested.

Significance: Our study provides evidence the revised *Salmonella* BAM shell egg preenrichment medium supports recovery of diverse and injured *Salmonella* cells to sufficient levels for detection by a rapid screening qPCR assay.

P3-171 Relative Effectiveness of Lactose Broth and Selected Buffered Preenrichment Media for the Detection of *Salmonella* in Artificially Contaminated Casein-based Powdered Infant Formula

ANDREW JACOBSON, Hua Wang, James Smiley, Melanie Butler and Thomas Hammack
U.S. Food and Drug Administration, College Park, MD

Introduction: Lactose Broth (LB) is the default preenrichment medium for the BAM *Salmonella* culture method, however, buffered media have been demonstrated to be more effective than LB for detection of *Salmonella* in high microbial load foods ($P < 0.05$). The relative effectiveness of LB and buffered preenrichments for the detection of *Salmonell*a in low moisture/low microbial foods is unknown.

Purpose: Compare LB and buffered preenrichments for detection of *Salmonella* in casein based powdered infant formula (PIF).

Methods: Artificially contaminated test portions of casein based PIF were analyzed using LB, Universal Preenrichment Broth (UPB), Buffered Peptone Water (BPW), and modified Buffered Peptone Water (mBPW) in each experimental trial. Pairwise comparisons of results from the analysis of contaminated PIF, analyzed using the 4 different preenrichment media, were evaluated using chi-square 2-tailed F-test statistical analysis ($P < 0.05$).

Results: Ten test portions were identified as positive for *S*. Enteritidis with LB; a value higher than those of 2, 1, and 0 for UPB, BPW, and mBPW, respectively ($P < 0.05$). LB was also statistically more effective than UPB and mBPW for the detection of *S*. Anatum in casein based PIF ($P < 0.05$), however, there was no statistical difference ($P > 0.05$) in the recovery of *S.* Anatum from PIF, between LB and BPW, although LB was arithmetically superior to BPW. The number of *S*. Anatum –positive test portions for LB, UPB, BPW, and mBPW were 20, 13, 15, and 13, respectively.

Significance: LB was more effective than UPB, BPW, and mBPW for the detection of *Salmonella* in casein based PIF ($P < 0.05$).

P3-172 Evaluation of Three Real-time PCR Methods for Detection of *Salmonella* in Allspice, Cinnamon, and Oregano

XIAOHONG DENG, Aparna Tatavarthy, Laila Ali, Lijun Hu, Thomas Hammack and Guodong Zhang
U.S. Food and Drug Administration, College Park, MD

Introduction: Detection of *Salmonella* in some spices by PCR is still difficult due to inhibitory substances in those spices.

Purpose: The purpose of this study was to compare the effectiveness of three real-time PCR platforms in detection of *Salmonella* in allspice, cinnamon, and oregano and to evaluate the efficiency of different DNA extraction methods.

Poster

Methods: Eighteen separate trials were conducted using two different cultivars from each spices (allspice, cinnamon, and oregano), inoculated with three *Salmonella* serotypes, Montevideo, Typhimurium, and Weltevreden. Inoculation levels ranged from 1.7 to 3.5 log CFU/25g. Overnight pre-enrichment cultures were used to extract DNA for PCR, using two different methods (boil lysis and the standard methods) for each PCR platforms. The three real-time PCR methods evaluated in the study were ABI-MicroSEQ®, FDA-PRLSW, and GeneDisc®.

Results: The detection rate of *Salmonella* by PCR methods from culture positive samples for all trials was 99.8% for MicroSEQ® (*n*=509) and 97.6% for both PRLSW (*n*=510) and GeneDisc® (*n*=509). The un-detected samples were mostly from cinnamon, indicating possible PCR inhibitors in cinnamon. Mean C_T values for cinnamon (26.86) were significantly higher than those for allspice (22.26) and oregano (20.15) (*P*<0.0001). The difference of mean C_T values between boil lysis and standard extraction was not significant for allspice (*P*=0.573) and oregano (*P*=0.064), but was significant for cinnamon (*P*<0.0001), with the standard extraction having lower Ct values.

Significance: The detection of *Salmonella* by all three PCR platforms was comparable with culture assay results. Selection between standard DNA extraction methods combined with different PCR methods or boil methods depended on the spice tested. Although the boil method is simple, when facing inhibitory problems, the standard DNA extraction protocol suggested by PCR kit manufacturers could perform better.

P3-173 Evaluation of *Salmonella* spp., *Salmonella* Enteritidis, and *Salmonella* Typhimurium Real-time PCR Kit Performance in Co-inoculated Poultry, Pork Meat, and Environmental Surface Samples

JANI HOLOPAINEN[1], Katharine Evans[2], David Crabtree[2] and Mikko Kauppinen[1]

[1]Thermo Fisher Scientific, Vantaa, Finland, [2]Thermo Fisher Scientific, Basingstoke, United Kingdom

Introduction: Thermo Scientific™ RapidFinder™ *Salmonella* Multiplex (Sp/SE/ST) PCR Kit is a real-time PCR based assay for the simultaneous detection and differentiation of *Salmonella* species, *Salmonella* Enteritidis, and *Salmonella* Typhimurium in poultry, pork, and environmental surface samples. The *Salmonella* Multiplex assay workflow includes a simple 15 minute sample lysis step followed by a 45 minute PCR run on the Applied Biosystems™ 7500 Fast or QuantStudio™ 5 Real-Time PCR instruments.

Purpose: The purpose of the study was to verify performance of the *Salmonella* Multiplex assay when poultry, pork, and environmental surface samples were co-inoculated with low levels of two *Salmonella* serovars *S*. Enteritidis and *S*. Typhimurium or *Salmonella* Kentucky. Presumptive results were compared to those obtained with the multiplex assay's culture confirmation and the USDA FSIS *Salmonella* reference method.

Methods: Ten poultry, pork, and surface swab samples were co-inoculated with low levels of two *Salmonella* serovars to achieve fractionally positive results in accordance with AOAC guidelines. All samples analyzed with the *Salmonella* Multiplex Kit were enriched in Buffered Peptone Water (ISO formulation) + 12mg/L Novobiocin at 41.5°C for 14-16 hours, lysed, tested, and analyzed according to the protocol described in the instructions for use. Presumptive positive results were confirmed using culture and serological confirmation methods. The reference method was performed according to the USDA FSIS guidelines.

Results: The *Salmonella* Multiplex assay method proved to be an accurate method even when samples were contaminated with multiple *Salmonella* serovars. The assay was 100% sensitive and 100% specific versus culture and serological confirmation. Detection of two serovars from a single sample was possible from poultry, pork, and surface swabs.

Significance: The study demonstrated that the RapidFinder™ *Salmonella* Multiplex assay is a rapid, easy-to-use, and reliable workflow for the detection of *Salmonella* spp., *S*. Enteritidis, and *S*. Typhimurium in poultry, pork, and environmental surface samples.

P3-174 Evaluation of Molecular *Salmonella* spp., *Salmonella* Enteritidis, and *Salmonella* Typhimurium Multiplex Assays Inclusivity and Exclusivity

JANI HOLOPAINEN[1], Katharine Evans[2], David Crabtree[2] and Mikko Kauppinen[1]

[1]Thermo Fisher Scientific, Vantaa, Finland, [2]Thermo Fisher Scientific, Basingstoke, United Kingdom

Introduction: Thermo Scientific™ RapidFinder™ *Salmonella* Multiplex (Sp/SE/ST) PCR Kit is a real-time PCR based assay for the simultaneous detection and differentiation of *Salmonella* species, *Salmonella* Enteritidis and *Salmonella* Typhimurium in poultry, pork, and environmental samples. The *Salmonella* Multiplex assay workflow includes a simple 15 minute sample lysis step followed by a 45 minute PCR run on the Applied Biosystems™ 7500 Fast or QuantStudio™ 5 Real-Time PCR instruments.

Purpose: The purpose of the study was to compare inclusivity and exclusivity of the *Salmonella* Multiplex assay against two commercially available real-time PCR assays designed to detect *S*. Enteritidis, only, or *S*. Enteritidis and *S*. Typhimurium concurrently from food samples.

Methods: Total of 196 *Salmonella* isolates including 60 *Salmonella* spp., 38 *S*. Enteritidis and 68 *S*. Typhimurium strains and 30 non-target strains were analyzed during the study. In addition, samples were lysed prior to PCR and analyzed with *Salmonella* Multiplex assay as detailed in the manual. The alternative assays were performed on the same panel of isolates according to the manufacturers' instructions.

Results: The *Salmonella* Multiplex assay proved to be the most accurate method of the three assays tested. Every assay tested returned a false positive result with *Salmonella* Blegdam, *Salmonella* Moscow and *Salmonella* Nitra isolates. The *Salmonella* Multiplex assay demonstrated superior specificity for all *S*. Enteritidis, *S*. Typhimurium and *Salmonella* species isolates tested; whereas, alternative assay A failed to detect one *S*. Enteritidis isolate and alternative assay B incorrectly identified *Salmonella* Dublin and *Salmonella* Gallinarum as *S*. Enteritidis.

Significance: The study demonstrated that the RapidFinder™ *Salmonella* Multiplex Kit provides a more reliable workflow for the detection and differentiation of *Salmonella* spp., *S*. Enteritidis, and *S*. Typhimurium than the other two commercially available real-time PCR assays.

P3-175 Comparative Study Between 3M™ Molecular Detection Assay 2 - *Salmonella* and ISO 6579 in Meat and Poultry Products from Thailand

WATCHARA JANWATCHARAGAN[1], Phumtreemas Srilert[1], Panida Pisaisawat[2], Nongnuch Promla[2], Wanida Mukkana[2], Saengrawee Jongvanich[2], Wipa Kongsakul[2] and Janejira Fuangpaiboon[2]

[1]Bureau of Quality Control of Livestock Products, Pathum –Thani, Thailand, [2]3M Thailand Limited, Bangkok, Thailand

Introduction: Salmonellosis is an infection caused by the *Salmonella* bacteria and is one of the leading causes of global foodborne disease. The detection of *Salmonella* spp. in exported meat and poultry products is of particular concern, partly due to varying importer regulations and specifications. To help alleviate economic losses that can arise from *Salmonella* contamination, the Thai meat and poultry industry requires pathogen detection methods that are fast, convenient, highly sensitive, highly specific, accurate and cost-effective.

Purpose: This study compared the performance of 3M™ Molecular Detection Assay 2 (MDA2) *Salmonella* with a modified reference method (ISO 6579:2002/Amd.1:2007) for the detection of *Salmonella* spp. in meat and poultry products from the central region of Thailand.

Methods: A total of 131 meat and poultry products obtained from slaughter houses, food processing plants, and fresh markets from the central region of Thailand were tested for *Salmonella* detection with both methods. Additionally, poultry samples (*n*=30) were inoculated with *Salmonella* Typhimurium ATCC 14028, at 1, 3, 5, 10 CFU/25g, to determine relative detection level, accuracy, sensitivity, and specificity.

Results: *Salmonella* was detected in 49 of the 131 collected samples. The 3M MDA2 *Salmonella* was able to detect as low as one CFU of *Salmonella* spp. in artificially contaminated poultry samples. In this study, the sensitivity of the 3M MDA2 *Salmonella* was calculated as 100%; specificity was 96.6%; and accuracy was 98.1%, considering both naturally and artificially contaminated samples.

Significance: The 3M MDA2 *Salmonella* delivered highly sensitive, highly accurate, and rapid detection of *Salmonella* spp. in meat and poultry products when compared to the modified ISO 6579 method.

P3-176 Evaluation of a Rapid Isothermal Amplification Method and Two Enrichment Protocols for *Salmonella* Detection in Frozen Strawberries

JORGE ADRIÁN MUNIZ FLORES[1], Dalia Lorena Rodríguez Herrera[1], Julia Perez-Montano[1], Gustavo González-González[2] and Ma. Ofelia Rodríguez-García[1]

[1]Universidad de Guadalajara, Guadalajara, Mexico, [2]3M Food Safety Mexico, Guadalajara, Mexico

Introduction: Fresh and refrigerated strawberries can be a pathogen vehicle; Survival of *Salmonella* in this product has been reported. Strawberries have low pH (3.0-3.9) and high amounts of phenolic compounds that may affect the recovery of *Salmonella* and inhibit molecular reactions. Sample preparation and enrichment stage are critical for *Salmonella* detection. Use of technologies such 3M™ Molecular Detection Assay provides real-time detection and next day results

Purpose: To evaluate the performance of a molecular based method with a single and two enrichment step for the recovery of *Salmonella* from frozen comminuted strawberries.

Methods: A *Salmonella* spp. cocktail (Typhimurium, Agona, Poona, Bardo and Saint Paul) was used to inoculate -samples of frozen comminuted strawberry by dipping. Three inoculum levels were used: high (50-100 CFU/sample), low (4-8 CFU/sample) and uninoculated level. Each 25g-sample was placed on a sterile bag and added with 225 mL of 3M™ Buffered Peptone Water-ISO and incubated 24h at 35°C (single enrichment protocol). Aliquots of 0.1 mL were transferred to 10 mL Rappaport Vassiliadis broth (RV-10) and incubated 24h at 41.5°C (two step enrichment protocol). All samples were culture confirmed according the FDA-BAM.

Results: No significant differences were found ($P>0.05$) when this rapid test were compared with the traditional method. Use of a single or double enrichment provides same recovery rate for *Salmonella* detection regardless the inoculum level ($P>0.05$). One uninoculated sample was reported as positive by the rapid method and confirmed by culture.

Significance: Use MDA assay with a single enrichment step for detection of *Salmonella* on frozen and comminuted strawberries provide fast and accurate results that are equivalent to the traditional method.

P3-177 Single-step Enrichment Followed by Real-time PCR Detection of Low Levels of Sub-lethally Injured *Salmonella* in Low-moisture Ready-to-Eat Foods

SERGIY OLISHEVSKYY[1], Cathy St-Laurent[1], Anne Hellmer[1], Melissa Buzinhani[1] and Michael Giuffre[2]

[1]FoodChek Laboratories Inc., Saint-Hyacinthe, QC, Canada, [2]FoodChek Systems Inc., Calgary, AB, Canada

Introduction: Low-moisture foods form an integral part of the modern human diet. Despite the fact that *Salmonella* cannot grow in low-moisture environment, these bacteria remain viable at low levels for extended periods of time and present a major cause of reported foodborne illness outbreaks related to the consumption of foods with low-water activity.

Purpose: The objective of this study was to optimize and validate application of alternative single-step enrichment to detect low levels of sub-lethally injured *Salmonella* with a real time PCR assay in low-moisture ready-to-eat foods.

Methods: Peanut butter (25 g), raisin (25 g) and raw almond (375 g) samples were artificially contaminated with different sublethally injured by heating or desiccation *Salmonella* strains and stabilized for 14 days prior to testing. For each matrix, five uncontaminated, 20 low level and five high level contaminated samples were enriched at 35°C in 75-750 mL Actero™ *Listeria* Enrichment Media, and then processed with the DuPont™ BAX® System Real-Time PCR Assay for *Salmonella* to compare with the samples tested according to the US FDA BAM 5 reference method.

Additionally, efficacy of the alternative method was confirmed with various low-moisture ready-to-eat foods closely related to the validated matrices. A total of 505 samples of 25 g each, including dried or dehydrated fruits such as apricot, apple, date and cranberry; nuts such as cashew, walnut and pecan; and natural, crunchy and honey peanut butter were artificially contaminated with different levels (0.2-10 MPN/sample) of sublethally injured *Salmonella* and tested using the alternative method.

Results: According to the Probability of Detection, the alternative method showed equivalent performance to the reference method. No false positive or false negative results were observed.

Significance: The alternative method offers the capability of detecting *Salmonella* in low-moisture ready-to-eat foods after only 16 hours of enrichment, thereby significantly reducing presumptive reporting time in comparison with the reference method.

P3-178 Rapid Detection of *Salmonella* in Raw Chicken Breast Using Real-time PCR Combined with Immunomagnetic Separation and Whole Genome Amplification

JI-YEON HYEON and Xiangyu (Sean-U)

Deng

University of Georgia, Center for Food Safety, Griffin, GA

Introduction: Nontyphoidal *Salmonella enterica* is one of the leading causes of foodborne illness worldwide, accounting for more than one million cases in the U.S each year. A number of real-time PCR-based detection for *Salmonella* have been developed; due to the low abundance and uneven distribution of *Salmonella* in food, they are typically preceded by enrichment, which constitutes a bottleneck to enabling same-day analysis without compromising detection sensitivity.

Purpose: We developed a real-time PCR-based detection combined with targeted cell capture by immunomagnetic separation (IMS) and highly efficient amplification of genomic DNA by multiple displacement amplification (MDA) for *Salmonella enterica* Serotype Enteritidis (SE) from chicken breast.

Methods: The limits of detection (LOD) of IMS-MDA real-time PCR were determined in artificially SE-contaminated chicken breast (*n*=18) after 0, 2, and 4-hour enrichment. In addition, the equivalence of this method in detecting refrigeration-stressed SE on chicken breast (*n*=90) was assessed by comparing with conventional real-time PCR and culture-based approach.

Results: The LODs of IMS-MDA real-time PCR for detecting SE in chicken meat were 10 CFU/g, 1 CFU/g, and 0.1 CFU/g after 0, 2, and 4-hour enrichment, respectively. The detection rate of IMS-MDA real-time PCR appeared to be almost on par with that of culture-based detection. *Salmonella* was detected at medium (1 CFU/g) and high (10 CFU/g) inoculum levels in 27 and 29 out of 30 samples after 4-h enrichment, respectively, and showed no statistical difference ($P > 0.05$) at the low (0.1 CFU/g) inoculum level. In addition, IMS-MDA real-time PCR yielded significantly more positive results compared to conventional real-time PCR with statistical difference ($P < 0.05$).

Significance: We demonstrated the potential of IMS-MDA real-time PCR as a rapid, sensitive, and affordable method for detecting *Salmonella* in food. The successful application of this method suggested that this technique may be used for other pathogens and food samples.

P3-179 An Electrochemical Aptasensor for Rapid Detection of *Salmonella* Typhimurium in Poultry Based on the Bifunctional Magnetic Nanocomposites

RONGHUI WANG[1], Meng Xu[1], Jianhan Lin[2], Ming Liao[3], Michael Kidd[1] and Yanbin Li[4]

[1]University of Arkansas, Fayetteville, AR, [2]China Agricultural University, Beijing, China, [3]South China Agricultural University, Guangzhou, China, [4]University of Arkansas, Department of Biological and Agricultural Engineering, Fayetteville, AR

Introduction: *Salmonella* Typhimurium is one of the most commonly identified foodborne pathogens for humans and animals, which pose a threat to human health and cause substantial economic cost to society. The development of a rapid, specific and sensitive method to detect *S.* Typhimurium is urgently needed for ensuring food safety.

Purpose: The objective of the present study was to develop an electrochemical aptasensor for rapid detection of *S.* Typhimurium, based on the magnetic bifunctional polydopamine (PDA) polymeric nanocomposites (PMNCs).

Methods: The core-shell magnetic beads (MBs)-glucose oxidase (GOx)@PDA PMNCs were first synthesized as the primary vehicle to support the further functionalization and to isolate and concentrate the target bacteria. Gold nanoparticles (AuNPs) were biochemically synthesized on the surface of PMNCs to adsorb anti-*Salmonella* aptamers. The 3, 3'-Dithiodipropionic acid di(N-hydroxysuccinimide ester (DTSP) was used to cross-link the AuNPs and the aptamers. The final product of the aptamers/GOx_{ext}/AuNPs/MBs-GOx@PDA PMNCs can carry the target bacteria and be magnetically attracted to the surface of the screen-printed interdigitated electrode (SP-IDME) for measurement. The redox probe containing glucose recognized the current changes caused by the attachment of *S.* Typhimurium cells.

Results: The synthesized aptamers/GOx_{ext}/AuNPs/MBs-GOx@PDA PMNCs was demonstrated to be able to generate current responses that were linearly related to the log concentration of *S.* Typhimurium. The detection limit of the developed aptasensor was 96 cfu *S.* Typhimurium/ml in pure culture, within one hour, without pre-enrichment procedures. Ongoing research will focus on the validation of the aptasensor using poultry samples.

Significance: The outcome of this study provided a bifunctional PMNCs to improve the sensitivity of the aptasensor due to the higher loading efficiency of biomaterials through PNMCs.

P3-180 Validation of a Cultural Method for the Detection and Isolation of *Salmonella* in Allspice, Cinnamon, and Oregano

GUODONG ZHANG, Laila Ali, Xiaohong Deng, Lijun Hu, Aparna Tatavarthy, Eric Brown and Thomas Hammack

U.S. Food and Drug Administration, College Park, MD

Introduction: Detection of *S*almonella in select spices remains a challenge due to their inherent antimicrobial properties.

Purpose: This study evaluated the effectiveness of a newly designed culture method for the detection of *Salmonella* in allspice, cinnamon, and oregano.

Methods: Two varieties of each spice were used in the study. Spices were inoculated with *Salmonella* ser. Montevideo, Typhimurium, or Weltevreden at 1.7 to 3.53 log CFU/25 g. Two sets of 20 samples (25 g) were analyzed using both the new method and the *Bacteriological Analytical Manual* (BAM) method. For the new method, 225ml of TSB preenrichment broth was added to 25g of allspice or cinnamon, in a sterile Whirl-Pak™ filter bag and vigorously shaken for 60 sec manually; the rinsate was transferred to a fresh sterile Whirl-Pak™bag and incubated at 35 ± 2°C for 24 ± 2 h. A 450ml aliquot of TSB was used for oregano because the leaves absorbed too much water. Eighteen separate trials were performed; each consisted of 40 samples.

Results: In the 18 separate trials, with cinnamon, allspice and oregano, the new method outperformed ($P < 0.05$) the BAM method in 9 trials. In the other 9 trials, there was no significant difference ($P > 0.05$) between the two methods. When combining data from six trials per spice, the new method detected 81, 90, and 83 positive; the FDA BAM detected 37, 60, and 50 positive of 120 test portions for cinnamon, oregano, and allspice, respectively. The differences were significant ($P < 0.05$).

Significance: In conclusion, the new method was more sensitive and effective and easier to operate compared to the current FDA BAM method for the detection of *Salmonella* in allspice, cinnamon and oregano.

P3-181 An Impedance Biosensor for Simultaneous Detection of Low Concentration of *Salmonella* Serogroups in Turkey Ready-to-Eat (RTE) Products

IBRAHEM JASIM[1], Amjed Abdullah[1], Zhenyu Shen[1], Shuping Zhang[1], Majed El-Dweik[2] and Mahmoud Almasri[1]

[1]University of Missouri Columbia, Columbia, MO, [2]Lincoln University Jefferson City, Jefferson City, MO

Introduction: Foodborne pathogens cause millions of infections annually in the U.S. and have the potential to cause enormous financial burden due to medical costs and product recalls. The current detection techniques are time consuming, e.g., Bacterial culture requires 3 to 5 days while PCR requires up 24 hours enrichment step.

Purpose: This study discusses the design, fabrication and testing of MEMS based impedance biosensors for onsite detection of three *Salmonella* serogroups simultaneously with high selectivity and sensitivity at low concentration within 30 minutes in poultry products.

Methods: The biosensor consists of three microfluidic channels, each includes (1) a region for concentrating and directing *Salmonella* cells toward the sensing microchannel using AC signal at specific frequency, (2) a region for bacteria detection based on impedance measurements. The three detection electrode surfaces were functionalized with three mixtures of premixed *Salmonella* antibody (type B, D and E)-cross-linker (Sulfo-LC-SPDP) solution, one for each channel and without causing any cross-contamination. The poultry samples were spiked with *Salmonella* type B and introduced via the sample inlet towards the focusing region. The antigen-antibody binding results in change in impedance. The biosensor was fabricated on a glass substrate using surface micromachining technology.

Results: The biosensor demonstrates capability to selectively detect *Salmonella* type B with a concentration as low as 7 cell/ml, and 10 cell/ml in turkey ready-to-eat (RTE) and raw chicken samples, respectively, with detection time of 30 minutes. The other two channels that were coated with *Salmonella* antibody type E and D have weak signals. The addition of focusing region has improved the signal strength by a factor ranged between 4 and 18.

Significance: Our biosensor allows poultry industry to test their products in-shift enabling them to delay the sanitation cycle e.g., from 1 day to 2 days increasing the production line's running hours.

P3-182 Detection of Low Levels of *Salmonella* Species in a Variety of Food Matrices Using the Rapidchek Select *Salmonella* Test Method

MEREDITH SUTZKO and Ann-Christine Allen
Romer Labs, Inc., Newark, DE

Introduction: Controlling the spread of *Salmonella* throughout the entire food supply chain has proven very complicated because of the many potential sources of contamination. Testing for the presence of *Salmonella* spp. has become a necessity for the food industry which creates a need for a flexible method that provides results in a timely accurate fashion and that has been validated on a range of both raw and processed foods.

Purpose: To validate the use of a commercially available test system for the detection of *Salmonella* species in a wide variety of food matrices.

Methods: 25g and 375g samples of the different food matrices were inoculated with a low level of *Salmonella*(Enteritidis ATCC 13076, Typhimurium ATCC 14028, or Montevideo ATCC 8387). The inoculum was held for 24 hours at 2 - 8°C for refrigerated foods. Samples for the test method were enriched in primary media, transferred to secondary media, and evaluated with test strips. All samples were confirmed using BGS and XLD agars.

Results: One hundred and twenty samples were analyzed in total for the 25 gram sample size and sixty samples were analyzed in total for the 375 gram sample size. All negative control samples were reported as negative (100% specificity).

Of the 110 low-level inoculated samples for the 25g sample size, eighty-two samples were detected with the test method and confirmed positive on XLD and BGS agars. For the 375g sample size, thirty-nine of 50 low-level inoculated samples were detected with the method and confirmed on selective agar plates. There were no false positive or false negative test results reported (100% sensitivity). The Chi-square for the results obtained by the test strip method and the cultural method was 0.00 (P=1.000).

Significance: The test system has been validated for use in several diverse sample matrices at 25g and 375g sample sizes.

P3-183 Comparative Evaluation of Two Multiplex Real-time Quantitative PCR (qPCR) for Detection of *Salmonella* spp. and *S.* Enteritidis in Pooled Egg Preenrichment Samples

GINA RYAN, Melanie Butler, Anna Laasri and Thomas Hammack
U.S. Food and Drug Administration, College Park, MD

Introduction: Foodborne pathogens, *Salmonella enterica* serotypes Enteritidis (SE) is a major problem in shell egg contamination. Current standards for validating qPCR assays for SE inclusivity require testing 100 strains at high concentration levels (LOD + 2 logs).

Purpose: Comparative evaluation of two FDA-developed multiplex SE qPCR assays for performance under challenging conditions (e.g., low contamination levels, egg-preenrichment background, divergent SE strains).

Methods: Two previously validated SE assays were evaluated: prot6E-qPCR (targets invA and prot6E genes) and safA-qPCR (targets ttrA and safA genes). A total of 350 *Salmonella* strains, including 100 diverse serotypes (FDA SAFE collection) and 250 *S*. Enteritidis from egg and poultry-associated sources were tested for inclusivity at three levels (LOD, LOD+1 log and LOD+2 logs) in pure isolate broth culture or egg preenrichment samples.

Results: At the highest level tested, all strains were correctly detected with safA-qPCR (both probes) and prot6E-qPCR (invA probe), while false negative results (29/250 SE) were obtained with prot6E-qPCR (prot6e probe). Assay performance for inclusivity was significantly reduced at lower contamination levels and in matrix background, though the extent varied widely for both assays and individual probes. Overall, differences in SE-qPCR assays ability to detect diverse *Salmonella* strains under challenging conditions may not be apparent using current validation guidelines.

Significance: Our data showed current validation standards (i.e., detection at LOD+2 logs) may not be sufficient to accurately predict assay performance under sub-optimal conditions (i.e., near the LOD, diverse strains, food matrix background) encountered in food environments.

P3-184 Evaluation of Several Elements in the Environmental Sampling of *Listeria* spp. from Stainless Steel Surface

Ishani Sheth, **FENGMIN LI**, Hee jin Kwon, Antonie De Jesus, Thomas Hammack, Karen Jinneman and Yi Chen
U.S. Food and Drug Administration, College Park, MD

Introduction: *Listeria monocytogenes* causes severe foodborne illness,listeriosis, which primarily affects pregnant women, newborns, elderly, and adults with weakened immune systems and has a case-fatality rate of around 20%. Currently, there are no validated devices and methods for environmental testing of this pathogen by FDA.

Purpose: This project was focused on the evaluation of several elements in the environmental sampling for *Listeria* spp.: sampling device, storage time after sampling, volume of enrichment broth.

Methods: *Listeria monocytogenes* was cultured overnight in Brain Heart Infusion (BHI) broth, diluted to proper concentrations, and then dried at room temperature overnight on stainless steel surface. The evaluation followed AOAC microbial method validation guidelines, comparing fractional positive results generated by each variable. The method described in FDA's *Bacteriological Analytical Manual* (BAM) was followed in the whole process. Different materials of swabs (cotton, polyester, and polyurethane) and sponges (cellulose and polyurethane) used for collecting *L. monocytogenes* were compared. After sampling, three storage conditions of the samples were compared: 2 hours at room temperature, 24 h at 4°C, 48 h at 4°C. Two different enrichment volumes were evaluated: 90 ml and 225 ml.

Results: All experiments generated fractional positive results required by the AOAC guidelines. The 3 storage conditions after sampling (2 hours at room temperature, 24 h and 48 hours at 4°C) generated statistically equivalent results. The two enrichment volumes (90ml and 225ml), also, generated statistically equivalent results. Different materials of swabs and sponges generated statistically equivalent results as well. Statistical analyses were performed by probability of detection (POD) analysis according to AOAC guidelines, as well as Fisher's Exact method.

Significance: This study contributed information that can be used to develop and optimize a complete procedure for environmental sampling of *Listeria* spp.

P3-185 The Survival and Transmission of Aerosolized *Listeria* Species

CALVIN WALDRON, Joseph Eifert, Linsey Marr, Andrew Neilson and Robert Williams
Virginia Tech, Blacksburg, VA

Introduction: Bioaerosol control in food production environments is important because of the potential for contamination to foods and food contact surfaces. The physical environment of a processing facility can affect the transmission and viability of *Listeria* (growth and death of cells) or other bacteria.

Purpose: The purpose of this work was to explore the ability of *Listeria innocua* to contaminate a surface after the organisms become aerosolized. Environmental factors including relative humidity, distance from source, and surface wetness that may influence the ability of the organisms to survive and attach to surfaces were explored.

Methods: *Listeria innocua* was nebulized into a biosafety chamber (~5 log CFU in 1 ml), and the environment was manipulated and contained within this chamber with a combination of four durations and two humidity levels (83% and 65%). Oxford agar media, stainless steel coupons and polyethylene coupons were placed in the chamber at three distances from the nebulizer. An Airport MD8 Air Sampler was used to sample the air, and the recovery of *Listeria* after 7, 12, 22 and 42 minutes was determined.

Results: The three sample surfaces yielded a greater mean recovery of 2.7 cells/cm^2 at 83% humidity compared to 0.45 cells/cm^2 at 65% humidity. Recovery from air was significantly higher (2.2 mean cells/L) at 7 or 12 min after nebulization (83% humidity) compared to other time/humidity combinations (0.42 mean cells/L).

Significance: Understanding the survival rate of aerosolized *Listeria* and the time interval that these aerosolized bacteria can still colonize a food contact surface will enhance our efforts to prevent transmission. If *Listeria monocytogenes* behaves in same manner as *Listeria innocua* under similar conditions, correlations can be made. The food industry could apply proper precautions when either organism is detected.

P3-186 Development of a Test Strip-based Method for the Detection of Group B *Salmonella* in Poultry House Environmental and Raw Poultry Samples

MARK MULDOON, Vera Gonzalez, Randy Bechard, Ann-Christine Allen and Meredith Sutzko
Romer Labs, Inc., Newark, DE

Introduction: *Salmonella* Group B serovars such as Typhimurium and Heidelberg are important human pathogens associated with food poisoning outbreaks. A rapid method for monitoring these serovars at both the live production and food processing stages could help in controlling their presence in food products. In order to aid in monitoring Group B *Salmonella*, we developed a rapid, lateral flow test strip-based method for the detection of Group B *Salmonella* in poultry house environmental and raw poultry samples.

Purpose: The purpose of this study was to develop a rapid, test strip-based method for the detection of Group B *Salmonella* in poultry house environmental and raw poultry samples.

Methods: Monoclonal and polyclonal antibodies were developed against Group B *Salmonella* and incorporated into a lateral flow test strip. The test strip was characterized against a panel of 71 *Salmonella* spp. strains representing 26 serogroups including 24 Group B strains. The test strip was coupled to a bacteriophage-based enrichment method and used in a spiked poultry house environmental sample study and a naturally-contaminated raw ground chicken study.

Results: The test strip gave 100% sensitivity and specificity in the Inclusivity / Exclusivity study. All *Salmonella* Group B strains were detected and none of the non-Group B strains were detected. For both the spiked poultry house environmental sample study and the naturally-contaminated raw ground chicken study, the method gave 100% sensitivity and specificity. For both sample types, all results from the test strip method culturally-confirmed (n=90). The Chi-square for the results obtained by the test strip method and the cultural method was 0.000 (P>0.999). Probability of Detection (POD) analysis showed no significant differences between the test method and the cultural method.

Significance: The new test method should provide the users with a rapid and reliable tool for monitoring and controlling *Salmonella* Group B serovars in live production environments and in food product.

P3-187 Application of RapidChek® *Listeria monocytogenes* Test System for the Detection of *Listeria monocytogenes* in a Variety of Foods and Environmental Surfaces

MARK MULDOON, Gregory Juck, Vera Gonzalez and Meredith Sutzko
Romer Labs, Inc., Newark, DE

Introduction: The USDA-FSIS *Listeria* Control Program requires that some RTE food processing facilities withhold the release of product pending *Listeria* test results. In order to reduce the cost of holding product, we developed a rapid, 44 to 48 h, lateral flow test strip-based method for the detection of *Listeria monocytogenes* in several food types and environmental surfaces.

Purpose: The purpose of this study was to verify the performance of a 44-48 h, test strip-based method for the detection of *Listeria monocytogenes* in several food types and environmental surfaces.

Methods: For each food or environmental surface type (14), a set of 5 non-spiked and 20 low level-spiked samples were tested by the new lateral flow test strip method at 44 to 48 h using RapidChek NextDay media incubated at 30°C. All samples were culturally confirmed. Low level spike levels ranged from 1 to 4 CFU per analytical unit (25 g).

Results: A total of 280 low-level spiked samples were tested by the test strip method followed by cultural confirmation. The test strip method gave 212 presumptive positive results and the cultural method gave 211. There were 2 false positives and 1 false negative by the test strip method. All non-spiked samples were negative for *Listeria monocytogenes*. The method gave a sensitivity of 99.5% and a specificity of 98.5%. The overall Chi square was 0.000 (P> 0.999). Probability of Detection (POD) analysis showed no significant differences between the test method and the cultural method.

Significance: This new test strip method should provide the end user with a rapid and reliable tool for monitoring and controlling *Listeria monocytogenes* in a variety of foods and environmental surfaces. Since it uses the same enrichment broth as the RapidChek *Listeria* spp. NextDay method, when used in combination with that method, it should provide valuable additional information to the end user for the control of *Listeria monocytogenes* in food and in the food processing environment.

P3-188 Evaluating Alternative Methods for the Detection of *Listeria monocytogenes* from Medical Nutrition Samples

KATHARINE EVANS, Emma Scopes and David Crabtree
Thermo Fisher Scientific, Basingstoke, United Kingdom

Introduction: Nutricia manufacture a broad range of medical nutrition products, including matrices inhibitory to bacterial growth, which are routinely tested for the presence of *Listeria monocytogenes* using an alternative culture method. A method with a shorter time to result for detection of this pathogen was desirable as the ISO method provides a time to negative result in 5 days. The Thermo Scientific™ SureTect™ *Listeria monocytogenes* PCR Assay is validated for the detection of *L. monocytogenes* from all food types and production environment samples via AOAC-RI PTM and NF Validation by AFNOR Certification, giving a time to negative result in 24-28 hours.

Purpose: To assess the suitability of the PCR assay for use with a range of medical nutrition products while maintaining a short time to result.

Methods: Nine medical nutrition samples were inoculated with 4.3 to 5.0 CFU *L. monocytogenes* then enriched and tested using the PCR assay and results compared against the ISO 11290-1:1996 method. Samples found to be inhibitory to growth of *L. monocytogenes* were tested with two alternative methods: a modified PCR method that included a non-selective regrowth following primary enrichment; and a rapid culture method.

Results: The PCR assay and ISO 11290-1:1996 method successfully detected *L. monocytogenes* from seven of nine samples tested. The remaining two samples were found to be inhibitory to growth of *L. monocytogenes* along with a further four samples identified by Nutricia. The modified PCR method detected *L. monocytogenes* from four of six inhibitory samples tested. The remaining two inhibitory samples were tested with the rapid culture method, achieving 100% sensitivity.

Significance: Alternative PCR methods to detect *L. monocytogenes* with short time to result (24 hours–4 days) were identified for all medical nutrition products, including samples inhibitory to growth which prevented detection of *L. monocytogenes* with the 5-day ISO culture based method.

P3-189 No Influence of Selective Growth Media on the Identification of the Foodborne Pathogens by MALDI-TOF MS

Marian Awad, **DANIÈLE SOHIER** and Markus Kostrzewa
Bruker Daltonics, Bremen, Germany

Introduction: Selective media are widely used to isolate and/or enumerate foodborn pathogens such as *Salmonella* spp., *Campylobacter* spp., *Cronobacter* spp., and *Listeria* spp. In many cases, a confirmation procedure is required; and MALDI-Tof Mass Spectrometry (MS) is one of them.

Purpose: The objective of this project is to evaluate the ability of MALDI-TOF MS to identify and confirm isolates directly from widely used selective agars, i.e. standard and proprietary media.

Methods: The effect of the growth medium on the generated MALDI-TOF MS Profile Spectra and its consequences for species identification were determined by using a set of relevant target and non-target strains. The following selective agars were tested: XLD, BGA, ASAP, Rapid'*Salmonella*, ESIA, DFI, mCCDA, Campy Cefex, CampyFood Agar, Rapid'*Campylobacter*, Ottaviani & Agosti, Oxford, Palcam, and Rapid L.mono. The isolates were plotted onto reusable and disposable targets. A HCCA matrix based sample preparation with and without formic acid (70%) overlaid was used for all the tested strains; an extraction procedure was also run for *Listeria* spp. confirmation. The MSPs were acquired and analyzed with the Maldi biotyper complete solution.

Results: More than 630 Spectra were generated. All the tested isolate were correctly identified, whatever the tested selective agars, targets, and sample preparations. No bias was observed. A subtyping module is also available to be used for *Listeria* spp. identification and it allows the use of the simplest sample preparation protocol consisting of the overlaid form of the single HCCA matrix, only.

Significance: There was no influence of selective growth media on the identification and, thus, confirmation of the tested foodborn pathogens by MALDI-Tof MS. Indeed, no culture step on a non-selective agar was required prior confirmation, and the simplest and quickest sample preparation was used.

P3-190 Performance Evaluation of 3M™ Molecular Detection Assay 2 for Rapid Detection of *Listeria monocytogenes* in Brazilian Meat Matrices

VANESSA TSUHAKO[1], Sandra Heidtmann[2], Adriana Bovo[2] and Analice Espeleta[2]
[1]3M Brasil, Sumare, Brazil, [2]Meat Industry, Jundiai, Brazil

Introduction: Rapid *Listeria monocytogenes* detection is critical to guarantee food safety and to protect food companies brands, as this pathogen contamination can result in public health problems and economic losses.

Purpose: To verify the specificity and sensitivity of 3M™Molecular Detection Assay 2 – *Listeria monocytogenes* (AOAC OMA 2016.08) for a variery of brazilian meat matrices compared to ISO 11290-1 and 11290-2; 2004.

Methods: A total of 104 samples (raw chicken (*n*=20), frozen ready to eat lasagna (*n*=15), 2 types of sausage (*n*=35), breaded chicken (*n*=15) and hamburger (*n*=19)) were artificially contaminated with *L. monocytogenes* and analyzed with both methods. Each group was divided into a) uninoculated, artificially contaminated with b) 7.6 and c) 18 CFU/sample and d) also with a population of *E. aerogenes* (260 CFU/sample) as an interference microorganism.

Results: Compared to the traditional method, specificity and sensitivity of the molecular method was 100% and 92.5% respectively. In all samples that presented discrepant results between methods, the molecular method had a better performance than the reference method utilized in this study.

Significance: The alternative molecular method enabled reliable, rapid and automated detection of *L. monocytogenes* in meat samples.

P3-191 Detection of *Listeria* in Probiotic Cultures

JOSEPHINE D. GREVE, Benjamin S. Shannon and J. David Legan
Covance Food Solutions, Madison, WI

Introduction: Consumption of probiotics is increasingly popular. The World Health Organization defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". There is a substantial body of literature describing the reduction of high *Listeria* counts in the presence of competing organisms. However, no literature describes effective methods for detecting low levels of *Listeria* in probiotic cultures, suggesting that food-safety monitoring of probiotics may be difficult.

Purpose: This study set out to establish the detectability of *Listeria* species in probiotic cultures and estimate the sensitivity of detection.

Methods: A dehydrated probiotic culture was spiked with known concentrations of *Listeria monocytogenes,* enriched in different broth media, and then tested using the Roka Atlas® *Listeria* species test protocol.

Poster

Results: No recovery was detected from probiotic samples spiked with approximately 30 CFU per 25 g and enriched at 1/10 and 1/100 in Demi-Fraser broth for 24 h at 35°C. Supplementation of the enrichment medium with antibiotics (Colistin Sulfate 10.0 mg/L with Moxalactam 20.0 mg/L; Clindamycin plus Erythromycin both at 8 mg/L: and all four together) did not lead to *Listeria* recovery. However, a 30 CFU/25 g spike was detectable after thermal pasteurization of the probiotic, showing that it was purely competitive pressure inhibiting *Listeria* recovery. A spike level as low as 100 CFU/25 g sample was detected following 24 h incubation at 1/100 in Demi-Fraser broth at 35°C, establishing that the limit of detection of *Listeria* in this type of matrix is between 30 and 100 CFU/25 g sample.

Significance: This study demonstrated the challenging nature of *Listeria* detection from concentrated probiotic cultures. However, the observed detection limit is likely still protective of public health given the extensive literature on death of *Listeria* in these environments.

P3-192 A Comparative Study of Enumeration Methods for *Listeria monocytogenes* on Naturally Contaminated Ready-to-Eat Foods

JEANINE BOULTER-BITZER

Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada

Introduction: *Listeria monocytogenes* is a foodborne pathogen that is the causative agent in the human disease listeriosis. In foods and in food processing environments, *L. monocytogenes* is exposed to various stressors that possibly decrease its viability in terms of culture recovery with standard laboratory enrichment methods. Highly selective culture media used to facilitate growth of *L. monocytogenes* over competitive background flora may impact recovery of sub-lethally injured cells.

Purpose: Since it is expected that healthy individuals ingest small numbers of this bacterium without suffering any apparent ill effects, some countries, including Canada, have established quantitative guidelines for *L. monocytogenes* in ready-to-eat (RTE) food products. This study compared two enumerative methods for *L. monocytogenes*; the Health Canada method MLFP-74, which is based on direct plating without enrichment recovery, and the Most Probable Number (MPN) method, which utilizes enrichment steps, following the Health Canada method MFHPB-30.

Methods: Forty-five RTE meat and nine RTE sprout samples obtained from regulatory surveillance programs that tested positive for *L. monocytogenes* with MFHPB-30 were enumerated using both methods.

Results: The MLFP-74 method, with a detection limit of 5 CFU/g, resulted in all samples tested having less than the minimum detection limit (<MDL) of *L. monocytogenes*. The MPN method, with a minimum detection limit set to <0.03 mpn/g found that eight of the 45 RTE meat samples had countable levels of *L. monocytogenes*, which ranged from 0.03 mpn/g to 2.9 mpn/g. All other meat sample results were <MDL. Five of the nine sprout samples had countable *L. monocytogenes* levels which ranged from 0.04 mpn/g to 0.43 mpn/g. All other results for sprouts were <MDL.

Significance: These naturally-contaminated samples contained low levels of *L. monocytogenes*. There was no notable difference in results obtained by the two enumerative methods. A comparison involving more highly contaminated products would offer a greater opportunity to assess the potential for recovery issues.

P3-193 Rapid Species-Specific Identification of *Listeria* Isolates Using Multiplex PCR

BRADLEY ZIEBELL[1], Kari Sweeney[2] and Deann Akins-Lewenthal[1]

[1]*Conagra Brands, Chicago, IL,* [2]*Conagra Brands, Omaha, NE*

Introduction: Standard commercially available test methods used by the food industry target either the genus *Listeria* or *Listeria monocytogenes,* directly. These results must be confirmed using culture methods. However, these confirmation methods are time consuming, require multiple steps, and cannot simultaneously differentiate between multiple species of *Listeria*.

Purpose: This study was performed to develop a novel multiplex PCR method that will provide rapid, identification of *Listeria* spp. within a single test.

Methods: Six species-specific primer sets targeting *hly*, *iap*, *lse24*, *smcL*, *Lwe1801,* and one genus-specific primer set targeting *23S rRNA* were used for simultaneous differentiation of *L. monocytogenes*, *L. innocua, L. grayi* subsp. *grayi*, L. *grayi* subsp. *murrayi*, *L. seeligeri*, *L. ivanovii*, *L. welshimeri*, and the genus *Listeria,* respectively. DNA extracted from 13 *Listeria* spp. ATCC strains and 64 unknown presumptive *Listeria* spp. isolates were tested by the multiplex PCR method to evaluate the efficiency of the selected primer sets. Species identification was determined by gel electrophoresis based on the designated size (bp) of the species-specific PCR product.

Results: The multiplex PCR method simultaneously amplified both a species and a genus specific PCR product per strain and successfully identified all 13 ATCC strains of *Listeria.* Additionally, this method identified 22 unknown isolates as *L. monocytogenes* (*hly* ~713 bp)*,* 42 isolates as *L. innocua* (*iap* ~975-987 bp), and as expected demonstrated that all 64 isolates were within the genus *Listeria* (*23S rRNA* ~77bp). Overall, the multiplex PCR method was efficient and 100% accurate for species-specific identification of *Listeria*.

Significance: This new multiplex PCR method offers rapid, identification of presumptive *Listeria* spp. isolates. This knowledge will enhance risk assessments and provide information regarding the prevalence and potential co-existence of each species of *Listeria* in food products and the food production environment.

P3-194 Real-time Monitoring of *Listeria* Species and *Listeria monocytogenes* Using Non-invasive Bioluminescence Growth Media

Brandon Katz, Paul Meighan and **DELIA CALDERON**

Hygiena, Camarillo, CA

Introduction: This study introduces a new technology from Hygiena, MicroSnap Surface Express,for real-time monitoring of *Listeria* species and *Listeria monocytogenes* from surfaces. Collected dried *Listeria* begin to produce light as they metabolise, indicating by the emission of light per bacteria a new method to both enumerate and detect at low CFUs.

Purpose: To demonstrate the performance of MicroSnap Surface Express *Listeria* for real-time detection of *Listeria* from environmental surfaces.

Methods: Stainless steel coupons were inoculated with 100uL of a mixture of 4 *Listeria* species (*monocytogenes*, welshmeri, innocua and ivanovii) in BPDW. The coupons were dried overnight and swabbed with MicroSnap Surface Express *Listeria* ; test devices were then incubated for 24 hours and measured using the EnSURE luminometer every hour. A duplicate set of coupons were swabbed and used as reference methods *Listeria* petrifilm and MOX agar.

Results: The survivability from the drying process was calculated by comparing the dried with added inoculum levels, this was determined to be 0.004%. The -1 dried dilution had a mean (*n*=5) CFU of 83550, -2 had 8350 CFU, -3 had 830 CFU and -4 37 CFU all other dilutions discovered no colonial growth. The assays started to show a positive RLU (mean blank plus 6 standard deviations) in the EnSURE at 8 hours for -1, -2 dilution was positive at 10 hours with -3 becoming positive at 12 hours and -4 becoming positive at 14 hours. This time of flight determination prove the *Listeria* bacteria are

actively metabolising the substrate and producing light when a threshold CFU is reached. The higher the CFU the quicker the determination of positive can be determined.

Significance: The ability to determine whether a surface is highly contaminated with *Listeria* in less than 24 hours revolutionizes modern *Listeria* environmental monitoring practices.

P3-195 Enrichment Dynamics of *Listeria monocytogenes* and the Associated Microbiome from Naturally Contaminated Ice Cream Linked to a Listeriosis Outbreak

Elizabeth Reed[1], James White[2], Eric Brown[1], Yi Chen[1], Andrea Ottesen[1] and **PADMINI RAMACHANDRAN**[1]

[1]U.S. Food and Drug Administration, College Park, MD, [2]Resphera Biosciences, Baltimore, MD

Introduction: Microbiota that co-enrich during efforts to recover pathogens interfere with efficient detection and recovery. Here we describe co-enriching microbiota from ice cream linked to an outbreak of listeriosis using protocols from: 1) the Food and Drug Administration (FDA), 2) the International Organization of Standardization (ISO), and 3) the United States Department of Agriculture (USDA).

Purpose: This study was conducted to optimize recovery of pathogens from enrichments for expedited trace-back of outbreak-associated strains.

Methods: Enrichment methods were followed according to the published protocols for each method. Aliquots were taken every 4 h throughout 48 h of incubation. DNA was extracted using the Qiagen Dneasy Kit according to the manufacturer's specifications. Shotgun libraries were prepared using Truseq, and 16S rRNA gene amplification targeted the V1/V2 region. Sequencing was performed on an Illumina Miseq. Taxonomic profiling was performed using Resphera Insight and the Genius bioinformatics software package from CosmosID.

Results: Dominant co-enriching bacterial taxa were identified as *Anoxybacillus* spp., *Geobacillus* spp., *Serratia* spp., *Pseudomonas* spp., *Erwinia* spp., and *Streptococcus* spp. *Anoxybacillus* spp. and *Geobacillus* spp. appear to competitively exclude *Listeria monocytogenes* (Lm) during early enrichment hours. Lm became dominant after 24 h in all three methods. Shotgun reads of Lm from hour 48 produced a draft genome of Lm with a similar tracking utility to pure culture isolates of Lm.

Significance: All three methods performed equally well for enrichment of Lm. The observation that *Anoxybacillus* spp. and *Geobacillus* spp. competitively excluded Lm provided novel information that can be used to further optimize enrichment formulations. Draft assembly of a genome of Lm from shotgun metagenomic data demonstrated the utility of this approach to expedite trace-back of outbreak-associated strains directly from enrichments.

P3-196 Evaluation of the GENE-UP® Assay for the Co-Detection of *Escherichia coli O157:H7 and Salmonella* spp. from Raw Ground Chicken

Vikrant Dutta[1], **JOHN MILLS**[2], Deborah Briese[2], Peter Ladell[2] and Stan Bailey[2]

[1]bioMérieux, Inc., Hazelwood, MO, [2]bioMerieux, Inc., Hazelwood, MO

Introduction: In the US, among the foodborne pathogens, non-typhoidal *Salmonella* and *Escherichia coli* O157:H7 (STEC O157) are responsible for an estimated 41,930 and 3,704 laboratory-confirmed episodes of illnesses. Most of the commercially available PCR systems utilize separate systems for the detection of these pathogens. Given the common niche occupied by these pathogens, and the overall similarity in their generation times, it would be useful for testing laboratories to have a common workflow that can be utilized for simultaneous detection of both pathogens. The GENE-UP™ *Salmonella* (SLM) and *E. coli* O157:H7 (ECO) assays are Fluorescence Resonance Energy Transfer (FRET) based real-time PCR that utilize a common workflow, including the sample preparation method.

Purpose: In this study, we have evaluated the ability of SLM and ECO to detect *Salmonella* and STEC O157 from a co-inoculated raw ground chicken matrix using the two mostly used enrichment media, BPW and mTSB.

Methods: The 25g raw ground chicken samples were co-inoculated with *Salmonella* and STEC O157, such that per media, *n*=20 samples were spiked at a fractional levels (0.25 – 0. 75cfu/test portion), while *n*=5 samples each were spiked at high levels (>2.5cfu/test portion) and no inoculation, respectively. All analytical outcomes were biochemically confirmed by the traditional reference method (USDA/FSIS MLG 4.09 and 5.09) and by an alternative method; including streaking onto bioMérieux's chromogenic agar, ASAP™ for *Salmonella* and ChromID™ EHEC for STEC O157.

Results: Statistically equivalent performance (95% CI) were observed upon {dPOD}, where alternative method demonstrated no significant differences between the presumptive and the confirmed results for both *Salmonella* and STEC O157 per BPW and mTSB [$dPOD_{cp}$: 0.0; LCL:-0.28; UCL: 0.28]

Significance: These data demonstrated that the GENE-UP™ system can be used for co-detection of *Salmonella* and STEC O157 and the common workflow significantly reduced the workload for laboratory testing personnel and enabled a quicker turnaround of results.

P3-197 Evaluation of the GENE-UP® *Listeria monocytogenes* (LMO) Assay for the Detection of *Listeria monocytogenes* in Foods

JOHN MILLS[1], Stan Bailey[1], Deborah Briese[1], Vikrant Dutta[2], Hari Dwivedi[2] and Ron Johnson[2]

[1]bioMerieux, Inc., Hazelwood, MO, [2]bioMérieux, Inc., Hazelwood, MO

Introduction: The GENE-UP® is a novel real-time PCR system for pathogen detection. This new PCR platform is highlighted by an easy to use workflow and overall flexibility.

Purpose: The PCR *Listeria monocytogenes* assay was evaluated according to the current AOAC validation guidelines. The evaluation consisted of the following studies: inclusivity/exclusivity, method comparison of 13 food matrices, lot-to-lot/stability and robustness. This poster presentation will focus on the food comparison portion of the AOAC validation study.

Methods: The alternative method was compared to the USDA/FSIS-MLG 8.09 *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry and Egg Products, and Environmental Sponges* (2013) for deli ham, deli roast beef, deli turkey and turkey hot dogs; to the FDA/BAM Chapter 10 *Detection and Enumeration of Listeria monocytogenes in Foods* (2011) for cooked shrimp, fresh spinach, mixed bagged salad, liquid whole egg and whey powder; and to the AOAC OMA 993.12 *Listeria monocytogenes in Milk and Dairy Products* for Mexican soft cheese and vanilla ice cream. All analytical outcomes were biochemically confirmed by both traditional reference method and by an alternative method, which included an additional streak to chromogenic Agar *Listeria* Ottavani and Agosti (ALOA) agar.

Results: In the method comparison study, the alternative method demonstrated no statistically significant differences between presumptive and confirmed results ($dPOD_{cp}$) or between candidate and reference method results ($dPOD_{c}$) for the food matrices tested.

Significance: These data demonstrate that the GENE-UP LMO assay is a suitable method for detecting *Listeria monocytogenes* in a variety foods. The method provides significant savings in terms of time and improved convenience when compared to reference methods.

Poster

P3-198 EN ISO 16140-2 Validation Study of the GENE-UP® PCR Method for the Detection of *Listeria* sp. in a Variety of Food and Environmental Samples

Olivier Mathia[1], Louisiane Giovanetti[2], **FABIENNE HAMON**[2], Patrice Chablain[3] and François Le Nestour[1]
[1]ISHA groupe Alpa, Massy, France, [2]bioMérieux, Grenoble, France, [3]bioMérieux, Marcy, France

Introduction: Detection of *Listeria* sp. can take at least five days using traditional method. Alternative rapid screening methods have been developed to obtain quicker results for food and environmental samples. bioMérieux has developed GENE-UP® LIS for the specific detection of *Listeria* species.

Purpose: During the validation study conducted by the ISHA (Alpa Group, Massy, FRANCE), a sensitivity study was performed comparing this alternative method to the ISO 11290-1/A1:2004 Reference method for the detection of *Listeria* in 6 categories of food and environmental samples. The objective was to compare the alternative method to the ISO 11290-1/A1:2004 Reference method for the detection of *Listeria* in food and environmental samples.

Methods: For all categories (including meat products, dairy products, seafood, vegetables, composite foods and environmental samples), 551 samples of 25g were tested by both methods. This new method consists in a single enrichment in LPT broth incubated 22h for food samples and 18h for environmental samples at 37°C. Then, DNA is extracted using mechanical lysis in a dedicated lysis tube: 20µL are introduced through a cap that does not require any tube or cap handling. Extracted DNA is used directly with freeze dried PCR reagents.

The PCR method is based on dual probe detection (Fluorescence Resonance Energy Transfer) allowing real time detection and melting curve analysis. The call is positive when it combines an amplification curve and a melting peak allowing a strong specificity of the test.

Results: Methods were evaluated following the new ISO 16140-2 guidelines. The GENE-UP® *Listeria* sp method shows similar results to the ISO 11290-1/A1:2004 Reference method for the detection of *Listeria.*

Significance: GENE-UP® *Listeria* sp method enables a reliable and rapid detection of *Listeria* spp. in a variety of food : 22h enrichment, including a user-friendly workflow.

P3-199 EN ISO 16140-2 Validation Study of the GENE-UP® *Salmonella* Method in a Variety of Food

Justine Baguet[1], Cécile Bernez[1], **FABIENNE HAMON**[2], Louisiane Giovanetti[2], Patrice Chablain[3] and Maryse Ranou[1]
[1]ADRIA Food Technology Institute, Quimper, France, [2]bioMérieux, Grenoble, France, [3]bioMérieux, Marcy, France

Introduction: Detection of *Salmonella* spp. can take at least three days using traditional method. Alternative rapid screening methods have been developed to obtain quicker results for food and environmental samples. bioMérieux has developed a universal PCR method on the GENE-UP® thermocycler for the detection of foodborne pathogen, including *Salmonella* spp.

Purpose: During the validation study conducted by the ADRIA Développement (Quimper FRANCE), a sensitivity study was performed comparing this alternative method to the ISO 6579 Reference method for the detection of *Salmonella* spp. in seven categories of food samples.

Methods: Two protocols have been developed for same day, next day detection, and larger sample size. For next day detection, 333 samples of 25g (including ready-to-eat/ready-to-reheat, meat products, milk and dairy products, vegetables, fruits and seafood, specific ingredients and food) were tested by both methods. For same day detection and larger sample size, 65 raw meat samples of 25g and 63 raw meat samples of 375g were tested. This new method consists of a single enrichment in BPW incubated 8 to 24h (depending on food) at 41.5°C. DNA is extracted using mechanical lysis in a dedicated lysis tube: 20µL are introduced through a cap that does not require any tube or cap handling. Amplification, based on dual probe detection, is performed directly with freeze dried PCR reagents. The call is positive when it combines an amplification curve and a melting peak allowing a strong specificity of the test.

Results: Methods were evaluated following the new ISO 16140-2 guidelines. The GENE-UP® *Salmonella* method showed similar results to the ISO 6579 Reference method for *Salmonella* spp. detection.

Significance: The GENE-UP® *Salmonella* method enabled reliable and rapid detection of *Salmonella* spp. in a variety of food using an 8 to 24h enrichment (depending of food category and size) and providing a user-friendly workflow.

P3-200 Evaluation of a Method Based on Loop Mediated Isothermal Amplification and Bioluminescence Technology for the Detection of Human Pathogens on Grape Tomatoes

GUSTAVO GONZALEZ GONZALEZ[1], Lucila Trigueros-Díaz[2], María Cristina Luquin-Rosas[2] and María del Carmen Tinajero-Arriola[2]
[1]3M FSD Mexico, Guadalajara, Mexico, [2]Grupo Cencon, Guadalajara, Mexico

Introduction: Outbreaks of foodborne pathogens have been associated with produce consumption; and tomatoes have been a common produce item involved in these outbreaks. The use of rapid and specific technologies for pathogen detection are key to food safety management.

Purpose: This investigation evaluated the performance of a loop mediated isothermal amplification method (LAMP) alternative method for detecting *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes* on grape tomatoes according to the Mexican Department of Health Standard.

Methods: Grape tomatoes were collected and held at 4°C up to 24h. Samples were spiked with 6±2CFU of each target microorganism (*n*=20/organism). Another set (*n*=20/organism) was inoculated with the target microorganism and 172±18CFU of a non-target organism; *Citrobacter freundi*, *Enterobacter aerogenes*, and *Listeria innocua* for *Salmonella* spp. analysis, *E. coli* O157, and *L. monocytogenes* tests, respectively. Three uninoculated samples/organism were used as a negative control. Each 25g portion was enriched with 225 mL of Buffered Peptone Water for *Salmonella* and *E. coli* O157:H7 and with Demi Fraser for *Listeria*. Enrichments were incubated at 37+1°C for 24h. Detection of *Salmonella* spp., *E. coli* O157, *Listeri*a spp., and *L. monocytogenes* was performed using the LAMP method. Samples were analyzed by two technicians. Repeatability, reproducibility, uncertainty, and limit of detection were determined according to the standard criteria.

Results: *Salmonella* spp., *Listeria* spp., and *E. coli* were detected in inoculated samples with and without a non-target microorganism. *Listeria monocytogenes* was detected in all inoculated samples; but for those also containing a non-target organism, four true negatives were determined. All uninoculated samples were negative. The alternative method met all the criteria to be considered as suitable for the pathogen recovery and detection in grape tomatoes.

Significance: Use of a LAMP based method for the detection human pathogens on fresh grape tomato provided a rapid and specific approach to monitor the presence of this pathogens and met Mexican standard requirements

Poster

P3-201 Simultaneous Enrichment *of Salmonella* spp., *Escherichia coli O157:H7*, and *Listeria monocytogenes* in Spices and Seafood

KIRSTEN HIRNEISEN[1], Venugopal Sathyamoorthy[2], Atin Datta[2], Richelle Richter[1] and Donna Williams-Hill[1]
[1]U.S. Food and Drug Administration, Irvine, CA, [2]U.S. Food and Drug Administration - CFSAN, Laurel, MD

Introduction: Rapid detection of *Salmonella* spp., *Escherichia coli*, and *Listeria monocytogenes* in foods is important for the prevention of foodborne illnesses. In accordance with the FDA Bacteriological Analytical Manual (BAM), different enrichment broths are used for different target pathogens and food matrices. Multipathogen detection assays for foodborne pathogen detection are desired for increased efficiency and reduced cost to the laboratory. In order to achieve this, a broth that can enrich for multiple target pathogens simultaneously is essential.

Purpose: The objective was to identify the best enrichment broth to simultaneously enrich *Salmonella* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* in seafood and spices for a multiplex qPCR detection assay.

Methods: *Salmonella* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* were co-inoculated in a 1:1:1 ratio into 25g of tuna or red pepper spice blended with 225g of a candidate enrichment broth. Candidate broths included BAM media such as Buffered *Listeria* Enrichment Broth (BLEB) and modified Buffered Peptone Water (mBPW), published research broths including SEL, an FDA Research Broth (BMW) and modifications of these broths. After 24h incubation at 35°C, multiplex qPCR was performed on DNA extracts for pathogen detection.

Results: All target pathogens were detected in tuna enrichment in all candidate broths. Variations in Ct values for *Salmonella* Typhimurium and *E. coli* O157:H7 between the candidate broths were significantly different (P <0.05). For example, *E. coli* O157:H7 Ct values ranged from 24.11±0.52 in mBPW+glucose to 36.1±6.25 in UPB (n=3). In red pepper, *L. monocytogenes* was not detected in BMW, Buffered BMW, mBPW and SSL broth enrichment. *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* had Ct values of 29.26±2.09, 28.59±2.07 and 35.85±0.86, respectively, in red pepper mBPW+glucose enrichment.

Significance: The results will impact regulatory microbiological methods for the detection of multiple microbial target pathogens from foods and will provide a less labor intensive means for pathogen screening.

P3-202 Independent Method Comparison Evaluation of the Biomerieux VIDAS® *Listeria monocytogenes Xpress* (LMX) to the Health Canada MFHPB-30 Reference Method

PATRICK BIRD, James Agin, Joe Benzinger, Erin Crowley, Alison DeShields and David Goins
Q Laboratories, Inc., Cincinnati, OH

Introduction: Among the food-borne pathogens, *Listeria monocytogenes* has the highest case fatality rate and remains a major threat for immunocompromised individuals. The broad adaptive attributes of *Listeria* spp. makes it a suitable contaminant for many different food items.

Purpose: The purpose of this study was to conduct a method comparison evaluation of the bioMérieux VIDAS® *Listeria monocytogenes* Xpress (LMX) to the Health Canada *Compendium of Analytical Methods,* Volume 2 MFHPB-30 *Isolation of Listeria monocytogenes and other Listeria spp. from foods and environmental samples* in order to obtain the "all foods" claim for detection of *Listeria monocytogenes*.

Methods: For the evaluation, test matrices included 16 food types from the following categories: Ready-to-Eat Meat and Poultry (n=4), Multi Ingredient Composite Ingredient Foods (n=3), Fish and Seafood Product (n=3), Fruit and Vegetable- Based Products (n=3), and Dairy Products (n=3). Each of these food types included either 25 gm and /or 125 gm test portions. Each food item was inoculated with *L. monocytogenes* such that n=20 were at fractional level (0.2-2 CFU/test portion), n=5 were at high level (2-5 CFU/test portion); n=5 were left uninoculated. All analytical outcomes were biochemically confirmed by both MFHPB-30 and by an alternative method using bioMérieux's chromogenic Agar *Listeria* Ottavani and Agosti (ALOA) agar.

Results: No statistically significant differences were observed between presumptive and confirmed results ($dPOD_{CP}$) or between candidate and reference method results ($dPOD_C$) for the food matrices tested. Sensitivity, specificity, false negative, false positive, and test efficacy were found to be 100%, 100%, <1.0, <1.0, and 100%, respectively for fractional and high inoculated samples. No *Listeria monocytogenes* was recovered from the uninoculated samples.

Significance: These data demonstrate that the LMX assay is equivalent to the Health Canada MFHPB-30 reference method and is suitable for detecting *Listeria monocytogenes* in all foods.

P3-203 An Independent Evaluation of the GENE-UP *Listeria* species Assay for the Detection of *Listeria* Species in Foods and Environmental Surfaces

PATRICK BIRD, Benjamin Bastin, Joe Benzinger, Erin Crowley, James Agin and David Goins
Q Laboratories, Inc., Cincinnati, OH

Introduction: The ability of *Listeria* has to survive in various climates, such as low temperature and high pH, can cause severe problems for food manufacturers as the organism can survive cleaning conditions and contaminate food commodities. Recent regulations have increased the need for *Listeria* sampling programs in most food manufacturing industries. There is a need for rapid and accurate assays that can quickly detect *Listeria* from environmental samples and food commodities. The GENE-UP™ *Listeria* species Assay utilizes Real-Time PCR technology to provide next day results for *Listeria* detection.

Purpose: To independently evaluate the alternative rapid method to the USDA-FSIS 8.09 method for deli ham (125g) and stainless steel environmental surface (4" x 4" test area), to the FDA/BAM Chapter 10 method for smoked salmon (25 g), and the AOAC 993.12 method for Mexican soft cheese (25 g) as part of the AOAC RI™ PTM validation process.

Methods: Using 30 unpaired samples for each matrix, 5 replicates were inoculated at a high inoculation level, 20 at a low inoculation level and evaluated along with 5 uninoculated control replicates. After sample enrichment in LPT Broth, test portions were evaluated by both the alternative and reference methods. Samples were confirmed following procedures outlined in the USDA/FSIS-MLG 8.09, FDA/BAM Chapter 10, AOAC 993.12, and bioMérieux' s alternative confirmation procedure.

Results: Results for the assay were compared to the MLG, FDA, and AOAC reference methods by POD statistical analysis. No statistically significant differences were observed between the new method and the reference methods in the 3 foods and 1 environmental surface.

Significance: The data from the study, demonstrates that the GENE-UP™ *Listeria* species Assay is a rapid and reliable method for the detection of *Listeria* species in the food matrices and stainless steel environmental surface (4" x 4" test area) analyzed.

Poster

P3-204 An Independent Evaluation of the GENE-UP *Listeria monocytogenes* Assay for the Detection of *Listeria monocytogenes* in Foods

PATRICK BIRD, Benjamin Bastin, Joe Benzinger, Erin Crowley, James Agin and David Goins
Q Laboratories, Inc., Cincinnati, OH

Introduction: The persistence of *Listeria monocytogenes* to survive in extreme food manufacturing environments can cause severe problems in finished product for manufacturers. The number of people infected by foodborne *L. monocytogenes* is less than other foodborne pathogens; however, outbreaks from *Listeria monocytogenes* can produce high mortality rates. Foods such as raw milk cheeses, pasteurized dairy products, smoked seafood, ready-to-eat deli meats and cantaloupe have been implicated in such outbreaks. The GENE-UP™ *Listeria monocytogenes* Assay is a Real-Time PCR assay designed to detect fluorescence at several wavelengths to allow for multi-target detection in the same reaction vessel. The assay uses dual Fluorescence Resonance Energy Transfer (FRET) hybridization probes to detect target analytes.

Purpose: To independently evaluate the alternative rapid method, to the USDA-FSIS 8.09 method for deli ham (25g), to the FDA/BAM Chapter 10 method for smoked salmon (25 g), and the AOAC 993.12 method for Mexican soft cheese (125 g) as part of the AOAC RI™ PTM validation process.

Methods: Using 30 unpaired samples for each matrix, 5 replicates were inoculated at a high inoculation level, 20 at a low inoculation level and evaluated along with 5 uninoculated control replicates. After sample enrichment in LPT Broth, test portions were evaluated by both the alternative and reference methods. Samples were confirmed following procedures outlined in the USDA/FSIS-MLG 8.09, FDA/BAM Chapter 10, AOAC 993.12, and bioMérieux's alternative confirmation procedure.

Results: Results for the assay were compared to the MLG, FDA, and AOAC reference methods by POD statistical analysis. No statistically significant differences were observed between the new method and the reference methods in the 3 foods.

Significance: The data from the study, demonstrates that the GENE-UP *Listeria monocytogenes* Assay is a rapid and reliable method for the detection of *Listeria monocytogenes* in the food matrices analyzed.

P3-205 An Independent Evaluation of the GENE-UP® EHEC Detection Method for the Detection of Non-O157 Shiga-toxin Producing *Escherichia coli* (STEC) and *Escherichia coli* O157:H7 in Foods

PATRICK BIRD, Benjamin Bastin, Joe Benzinger, Erin Crowley, James Agin and David Goins
Q Laboratories, Inc., Cincinnati, OH

Introduction: Most *Escherichia coli* are harmless; however, some shiga toxigenic *escherichia coli* (STEC) produce toxins (*stx1*, *stx2,* and *eae*) that can cause serious illness. The GENE-UP® EHEC Detection Method, which consists of three GENE-UP® tests, STEC (EH1), the GENE-UP® *E. coli* O157:H7 (ECO), and the GENE-UP® STEC-Top 6 (EH2), is designed to detect the virulence genes, and identify the top seven STEC strains. The assays utilize proprietary PCR technology to detect fluorescence at several wavelengths to allow for multi-target detection in the same reaction vessel. The resulting signal forms a real-time amplification curve after the PCR cycling program is completed; the PCR products are melted to determine the presence of the target DNA. The software uses both the amplification curve and the melt peak to make a positive or negative interpretation.

Purpose: This study was conducted to independently evaluate an alternative rapid method to the FDA/BAM Chapter 4A method for spinach (200g), the USDA/FSIS MLG 5.09 method for raw beef trim (375 g), and the USDA/FSIS MLG 5B.05 method for raw beef trim (375 g) and raw ground beef (25g and 375g) as part of the AOAC RI™ PTM validation process.

Methods: Using 30 unpaired samples, 5 replicates were evaluated at a high inoculation level, 20 at a low inoculation level, and 5 at an uninoculated control level. Samples were analyzed by the alternative method, and confirmed according to the reference method and a proprietary alternative procedure.

Results: Results for the assay were analyzed by POD statistical analysis. No statistically significant differences were observed between the new method and the reference methods.

Significance: The data from the study demonstrated that the GENE-UP® EHEC was a rapid and reliable method for the for the detection of non-O157 STEC Top 6 and *E. coli* O157:H7.

P3-206 Independent Evaluation of the Bio-Rad iQ-Check® *Salmonella* II Kit for the Detection of *Salmonella* Species in Select Foods and Environmental Surfaces

PATRICK BIRD, Benjamin Bastin, Joe Benzinger, Erin Crowley, James Agin and David Goins
Q Laboratories, Inc., Cincinnati, OH

Introduction: *Salmonella* has been implicated as a main cause of foodborne outbreaks and detection can be time consuming and expensive. Manufacturers require methods that are rapid and reliable for the detection of *Salmonella*. The Bio-Rad iQ-Check *Salmonella* II Kit is a test based on gene amplification and detection by the use of real-time PCR. Ready-to-use PCR reagents contain oligonucleotides specific for *Salmonella*, as well as DNA polymerase and nucleotides. The Free DNA Removal Solution (FDRS) step removes DNA from non-viable cells leaving viable bacteria to be detected by PCR, which reduces the amount of false positive results.

Purpose: This study independently evaluated the new method, comparing FDRS and without FDRS to FDA/BAM Chapter 5 method for milk chocolate (375 g), raw milk cheese (375 g), and stainless steel environmental surface (4" x 4" test area) and to the USDA/FSIS MLG 4.08 method for chicken carcass rinse (30 mL) as part of the AOAC RI™ PTM validation process.

Methods: Thirty unpaired samples were evaluated for each matrix (5 replicates were inoculated at a high inoculation level, 20 at a low inoculation level and 5 remained uninoculated (control) replicates). After sample enrichment, test portions were evaluated by both the new and reference methods. Samples were confirmed following procedures outlined in the FDA/BAM Chapter 5 and the USDA/FSIS-MLG 4.08.

Results: Results for the assay were compared to the FDA and MLG reference methods by POD statistical analysis. No statistically significant differences were observed between the new method and the reference methods in the three foods and one environmental surface.

Significance: The data from the study, within the statistical uncertainty, supported the product claims of the iQ-Check *Salmonella* II Kit and enhanced sensitivity in detection of *Salmonella* in the select food matrices and environmental surfaces analyzed.

P3-207 Validation of the 375 Gram Matrix Extension to Health Canada MFLP-38 Detection of *Salmonella* Species from All Foods and Environmental Surfaces Using the Bio-Rad iQ-Check® *Salmonella* II Kit

PATRICK BIRD, Benjamin Bastin, Joe Benzinger, Erin Crowley, James Agin and David Goins
Q Laboratories, Inc., Cincinnati, OH

Introduction: *Salmonella* is recognized as one of the most prevalent causes of food poisoning. A single foodborne outbreak can bring unimaginable economic losses to manufacturing companies; indicating a need for fast and reliable methods. The Bio-Rad iQ-Check *Salmonella* II Kit is based on gene amplification and detection by the use of real-time PCR. The assay contains ready-to-use PCR reagents containing oligonucleotides specific for *Salmonella*, as well as DNA polymerase and nucleotides. The assay, in combination with the Free DNA Removal Solution (FDRS) step, inhibits the amplification of target DNA from non-viable cells, which will aid in reducing the amount of false positive results.

Purpose: The objective of this study was to conduct a matrix extension evaluation of the Health Canada MFLP-38 method, with and without the FDRS step. The MFLP-38 was compared to the method for 375 gram test portions of milk chocolate, raw milk cheese, leafy green mix, custard filled pastry, smoked turkey breast, peanut butter, breaded chicken nuggets, vanilla ice cream, and stainless steel environmental surface (4" x 4" test area), as part of the Health Canada validation process.

Methods: Forty-five unpaired samples for each matrix were evaluated (20 replicates were inoculated at a high inoculation level, 20 at a low inoculation level, and 5 remained uninoculated). After sample enrichment, test portions were evaluated by both the new and reference methods. Samples were confirmed following procedures in the Health Canada MFHPB-20 reference method.

Results: Results for the assay were compared to the MFHPB-20 reference method by POD statistical analysis following Annex 4.4. No statistically significant differences were observed between the new method and the reference method in the eight foods and one environmental surface.

Significance: The data from the study, within the statistical uncertainty, supported the product claims of the iQ-Check *Salmonella* II Kit in detection of *Salmonella* in the select food matrices and environmental surfaces analyzed.

P3-208 Comparative Study: Extraction and Detection of Enteric Viruses in Soft Fruit

RACHEL RODRIGUEZ, Katja Schilling and Jacquelina Woods
U.S. Food and Drug Administration, Dauphin Island, AL

Introduction: Currently, multiple methodologies exist for the extraction and detection of enteric viruses such as norovirus (NoV) and hepatitis A virus (HAV) in soft fruit. With outbreaks occurring worldwide, it is important to have protocols that can accurately detect these viruses at low levels.

Purpose: The objective of this study was to compare the extraction and detection methodologies of enteric viruses in soft fruit using the ISO/DIS 15216-1 horizontal method for determination of HAV and NoV in food and the FDA validated soft fruit protocol.

Methods: For the ISO/DIS 15216-1 protocol, 25g of fresh blackberries and fresh blueberries were spiked with HAV in high (>10 PFU/g) and low (<5 PFU/g) concentrations. FDA validated methods utilized 50g of fresh blackberries and fresh blueberries spiked with HAV in high (>10 PFU/g) and low (<5 PFU/g) concentrations. Murine norovirus (MNV) was used as an extraction control for both protocols.

Results: With the ISO/DIS 15216-1 protocol, HAV was detected in fresh blackberries at the high (>10 PFU/g) levels but not in the low (<5 PFU/g) levels. In fresh blueberries, HAV was detected in both the high and low levels. With the FDA protocol, HAV was detected in fresh blackberries and fresh blueberries at both the high (>10 PFU/g) and low (<5 PFU/g) levels. The average extraction efficiency of MNV with the ISO/DIS 15216-1 protocol was 15% due to PCR inhibition and the average was 59% with the FDA protocol.

Significance: Rapid, sensitive methods to accurately detect viral pathogens in food samples are an integral part of outbreak and surveillance investigations. Extraction and detection of HAV in soft fruit utilizing the FDA validated method yields accurate results at high and low levels with minimal inhibition.

P3-209 Detection of Norovirus in Agricultural Water, Produce, and Hand-rinse Samples from Northern Mexico

JESSICA PRINCE-GUERRA[1], Anna M. Fabiszewski de Aceituno[2], Lee-Ann Jaykus[3], Zachary Marsh[1], Sharmila Talekar[1], Faith Bartz[1], Norma Heredia[4], Santos Garcia[4] and Juan Leon[1]
[1]Center for Global Safe Water, Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA, [2]RTI International, Research Triangle Park, NC, [3]North Carolina State University, Raleigh, NC, [4]Universidad Autonoma de Nuevo Leon, San Nicolas, Mexico

Introduction: From 1998-2008, viruses caused 42% of produce-associated outbreaks, with an estimated 3,500 due to norovirus. In the absence of standardized testing methods, few studies have been done to estimate the prevalence of norovirus in the agricultural environment.

Purpose: Pilot test an optimized detection method to identify both matrix inhibition and norovirus contamination on produce, hand-rinse, and agricultural water samples collected from northern Mexico.

Methods: From farms and packing facilities, composite rinses of equivalent surface area from cantaloupes (*n*=6 samples; 6 units/rinse), jalapeños (*n*=6 samples; 42 units/rinse), tomatoes (*n*=6 samples; 54 units/rinse), and hands (*n*=18 samples; 3 workers' hands/rinse) were collected in 0.1% peptone solution. Source and irrigation water samples (*n*=14 samples; triplicate 1.5L) were collected. A two-step virus concentration/elution procedure (1% BSA elution and 12% PEG precipitation) was followed by RNA extraction and RT-qPCRs (GI and GII) with an internal amplification control (IAC) to detect inhibition.

Results: An algorithm based on target and IAC cycle threshold (Ct) values was used to classify samples as presumptively positive, negative, undetermined, or inhibited. Thirty-nine of 50 samples had matrix-associated inhibition; repeating the RT-qPCR diluting template 1:4 removed inhibition in 69% of these. Using a Ct value cutoff <40, 12% (6/50) of the samples were presumptively positive, six undetermined, and 12 inhibited even after dilution. While at least one sample of each type (hand-rinse, water, produce) appeared positive, water had the highest prevalence (50%; 3/6). One cantaloupe rinsate was presumptively positive. Presumptively positive samples were mostly norovirus GII with varying Ct values (32.1 - 38.6). Sequence confirmation is pending.

Significance: This study provides evidence of norovirus RNA in the agricultural environment demonstrating hands and water as potential vehicles of contamination. The amount of RNA due to infectious virus remains unclear. This approach is useful for estimating norovirus RNA presence on various agricultural matrices.

P3-210 Comparison of Norovirus Surrogate Recovery Methods from Carpets

DAVID BUCKLEY, Angela Fraser, Guohui Huang and Xiuping Jiang
Clemson University, Clemson, SC

❖ ***Developing Scientist Competitor***

Introduction: Human noroviruses (HuNoV), a leading cause of foodborne disease, can survive on hard and soft surfaces. To determine its survival characteristics under various conditions, efficient recovery methods are needed. To date, no standardized methods are available for use with soft surfaces, such as carpet.

Purpose: We aimed to compare methods to recover a HuNoV surrogate, feline calicivirus, from carpets.

Methods: Wool and nylon carpet carriers (5 cm^2) were inoculated at a titer of ca. 6 log pfu/carrier with a HuNoV surrogate, feline calicivirus (FCV) strain F9, and recovered immediately or held at 30% RH and 25°C for 1 h. FCV was recovered by either bottle extraction (BE), macrofoam tipped swab (MS), or a wet vacuum system (WVS). BE and MS were tested with 2 buffers: 0.01M PBS + 0.02% Tween 80 or ¼ strength Ringers solution, whereas the WVS was only compatible with Butterfield's buffer. After recovery, BE and WVS solutions were concentrated via Amicon 30K tubes. Viruses were quantified via standard plaque assay.

Results: Recovery efficiency from nylon was 48.7 to 90.6%, 8.2 to 16.3%, and 11.8% when using BE, MS, and WVS, respectively, whereas recovery efficiency from wool was 33.3 to 76.8%, 46.0 to 74.0%, and 31.4% when using BE, MS, and WVS, respectively. Significantly more (P<0.05) infectious FCV was recovered when using BE and WVS compared to MS and more infectious FCV was recovered from wool than nylon carpets, excluding BE with ¼ strength Ringers solutions.

Significance: This is the first study to compare methods to recover viruses from carpets. BE and WVS exhibited higher recovery efficiencies compared to MS. However, each method demonstrates merit for use under different conditions, e.g. standard sanitizing testing and environmental monitoring.

P3-211 Withdrawn

P3-212 A Method for the Improved Detection of Aerosolized Influenza Viruses Using Impingers That Incorporate Anion Exchange Resin

JEFFREY CHANDLER[1], Joshua Schaeffer[2], Margaret Davidson[2], Sheryl Magzamen[2], Alma Perez-Mendez[3], Stephen Reynolds[2], Lawrence Goodridge[4], John Volckens[2], Alan Franklin[1], Susan Shriner[1] and Bledar Bisha[5]
[1]U.S. Department of Agriculture-APHIS-WS-NWRC, Fort Collins, CO, [2]Colorado State University, Fort Collins, CO, [3]Leprino Foods Company, Denver, CO, [4]McGill University, Montreal, QC, Canada, [5]University of Wyoming, Laramie, WY

Introduction: The important role of bioaerosols in influenza transmission is well established, as highlighted by the 2014-2015 outbreak of highly pathogenic avian influenza in U.S. poultry production. Accordingly, active sampling of bioaerosols for influenza (and other microorganisms) is suggested as an important mitigation tool.

Purpose: Here, we adapted an anion exchange resin-based method, initially developed to improve the detection of negatively-charged viruses in water, to impingement-based bioaerosol sampling of influenza viruses.

Methods: Type A and type B influenza viruses contained within the FluMist Quadrivalent vaccine were aerosolized to the standardized particle density of 5 mg/m^3 using a 6-jet collision nebulizer within a customized bioaerosol chamber. Aerosols were generated using 10-fold serial dilutions of viral inocula ranging in concentration from $10^{-2.5}$ to $10^{3.5}$ FFU/ml. For each experimental condition (tested in triplicate), two SKC BioSamplers containing 20 ml of PBS with and without 0.5 g of IRA-900 anion exchange resin were co-located within the chamber and calibrated to sample 500 liters of the bioaerosol. RNA was then isolated from the PBS used for impingement and from IRA-900 in preparation for real time reverse transcriptase PCR (RT-PCR) analyses.

Results: The anion exchange resin-based method improved detection of type A and type B influenza viruses by an average of 6.77× and 3.33× (P<0.05), respectively. Both type A and type B influenza viruses were detected more effectively at higher inoculum concentrations. Detection was improved by 9.55× for type A and 6.98× for type B influenza viruses when the viral inoculum used for nebulization was at a concentration of $10^{3.5}$FFU/ml.

Significance: A bioaerosol sampling method for influenza viruses was developed that allowed for improved molecular detection, was simple to perform, and adaptable to existing bioaerosol sampling equipment.

Poster

P3-213 Comparison of *Bdellovibrio bacterivorous* Viability and Predation Efficacy Following Different Delivery Methods and Storage Temperatures

DANIEL UNRUH and Sara Gragg
Kansas State University, Olathe, KS

❖ ***Developing Scientist Competitor***

Introduction: *Bdellovibrio bacteriovorus* is a non-pathogenic microbe that preys on Gram-negative bacteria. Knowledge of its application for the food industry, particularly at temperatures at which high-risk foods are stored, is limited.

Purpose: Temperature and delivery ("plug" versus "spin") methods were evaluated to determine *B. bacteriovorus* viability and predatory activity in the presence of *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC).

Methods: Frozen *B. bacteriovorus* was spotted onto YPSC agar with non-pathogenic *E. coli* (ATCC 43827) as prey, and 5-mm diameter "plugs" were removed from the resultant plaque. Half of the plugs were suspended in a solution of HEPES buffer containing prey *E. coli* ("spin"), while the remaining plugs were added to flasks containing HEPES + STEC or *Salmonella* cocktails ("plug"). The "spin" treatments were allowed to shake for 48 h and then added to HEPES + STEC or *Salmonella* cocktails. All flasks were held at 4°C, 10°C, 22°C, or 29°C. STEC and *Salmonella* were enumerated at 1, 2, and 3 d of storage on MacConkey and Xylose-Lysine-Tergitol-4 agar, respectively, while *B. bacteriovorus* was enumerated at 2 and 3 d by plating on YPSC agar.

Results: Populations of *B. bacteriovorus* were significant for delivery*day (P=0.0202). *Salmonella* populations differed by temperature*delivery (P=0.0049) and were greater in "spin" at 22 and 29°C and greater in "plug" at 10°C (P<0.05). STEC populations differed by temperature*delivery (P=0.0078) and day*temp (P=0.0192) and it is worth noting that "plugs" resulted in higher STEC populations at 4 and 10°C (P<0.05).

Significance: Temperature and delivery methods impact how *B. bacteriovorus* preys on *Salmonella* and STEC *in vitro* with reduced predatory activity at temperatures at which many foods are stored. Future research should be conducted to better understand and optimize *B. bacteriovorus*'s preying capabilities in different food products.

P3-214 Development of an In Vitro Assay for the Determination of Pathogenicity of *Vibrio vulnificus*

JOEY MARCHANT-TAMBONE[1], Jessica Jones[2] and Paul Gulig[3]

[1]FDA Gulf Coast Seafood Laboratory, DAUPHIN ISLAND, AL, [2]FDA Gulf Coast Seafood Laboratory, Dauphin Island, AL, [3]University of Florida, College of Medicine, Gainesville, FL

Introduction: *Vibrio vulnificus* (Vv) is the leading cause of mortality in United States shellfish consumers. However, Vv is ubiquitous in the environment and not all Vv isolated from the environment are pathogenic. As no reliable markers of pathogenicity have been identified, there is no rapid method to differentiate pathogenic strains.

Purpose: A cytotoxicity assay may be able to provide insight into the potential pathogenicity of Vv isolated from the environment. A mouse model has been developed and is the standard used to evaluate pathogenicity of Vv strains. However, this is time-consuming and expensive and not feasible to use as a screening method. This goal of this study was to develop a cell-based method to evaluate pathogenicity and aid in the identification of genetic markers for virulence potential without having to use a live animal model.

Methods: Environmental and clinical Vv isolates were tested in a cytotoxicity assay using RAW 264.9 (RAW) cells and compared to results from the standard mouse bio-assay using the Pearson Correlation. Briefly, using an LDH-releasing assay per the manufacturer's protocol, a set of 27 Vv isolates from environmental and clinical sources were tested for their cytotoxic effect on the RAW cells.

Results: Mouse mortality ranged from 0% to 90% and cytotoxicity in RAW cells ranged from 18% to 257%. Although some background interference was observed with the cell assay, there was a statistically significant correlation between mouse mortality and RAW cytotoxicity ($r=0.811$, $P=8.96 \times 10^{-7}$).

Significance: The results indicated the potential for the RAW cell model to reflect mouse (and, presumably, human) virulence. Development of a reliable cell-based model to identify pathogenic Vv will aid in identification of virulence genes, which can be used to develop rapid, molecular screening methods.

P3-215 Withdrawn

P3-216 Zero-valent Iron-Biosand Filtration is Capable of Reducing Antimicrobial and Generic *E. coli* Concentrations in Unbuffered Conventionally Treated Reclaimed Water: A CONSERVE Project

PRACHI KULKARNI[1], Greg Raspanti[1], Anthony Bui[1], Rhodel Bradshaw[1], Eric Handy[2], Cary Coppock[2], Kalmia Kniel[3], Manan Sharma[2], Amir Sapkota[1] and Amy Sapkota[1]

[1]Maryland Institute for Applied Environmental Health, University of Maryland, School of Public Health, College Park, MD, [2]U.S. Department of Agriculture–ARS, Beltsville, MD, [3]University of Delaware, Newark, DE

❖ ***Developing Scientist Competitor***

Introduction: If conventionally treated reclaimed water (RW) is to be adopted as an alternative to freshwater irrigation, it is necessary to investigate reuse site-based mitigation options that can further reduce contaminants such as foodborne pathogens and pharmaceuticals and personal care products (PPCPs) that may persist in RW.

Purpose: To examine the efficacy of a zero-valent iron (ZVI)-biosand filter in removing residual PPCPs, specifically antimicrobials, and generic *Escherichia coli* (*E. coli*) present in RW.

Methods: Populations of generic *E. coli* and concentrations of 13 antimicrobials commonly found in RW were quantified using the most probable number (MPN) analysis and high performance-liquid chromatography-tandem mass spectrometry, respectively, in unbuffered chlorinated effluent from a tertiary treatment plant. Testing was conducted before and after filtration through a 50:50; v:v macro-scale ZVI-biosand filter over a two-month period using a greenhouse-based experiment simulating reuse site conditions. The antimicrobials included were ampicillin, oxacillin, penicillin G, ciprofloxacin, oxolinic acid, pipemidic acid, azithromycin, erythromycin, vancomycin, linezolid, sulfamethoxazole, tetracycline and triclocarban. ZVI-filtered RW was then used to irrigate growing lettuce plants in the greenhouse, and lettuce plants were analyzed for the presence of *E. coli* after multiple irrigation events.

Results: Significant (p-value <0.01) reductions in concentrations were observed after ZVI-biosand filtration for ciprofloxacin, oxolinic acid, pipemidic acid, azithromycin, erythromycin, penicillin, linezolid and vancomycin with 100% reduction achieved for erythromycin. Generic *E. coli* ranged from 0.36 to 9.3 MPN/100ml in RW and was reduced to between < 0.3 and 2.3 MPN/100ml after ZVI-biosand filtration. No *E. coli*was detected on lettuce after irrigation events.

Significance: This greenhouse-based experiment shows promising results for providing a safe alternative to freshwater irrigation by reducing both *E. coli* populations and potential exposure to antimicrobials in reclaimed water. This study is part of a three-year effort to explore next-generation mitigation treatments to improve irrigation water quality (CONSERVE).

P3-217 Comparison of Two Methods for Enumeration of Total Fecal Coliforms and Generic *Escherichia coli*, and Their Ability to Predict Pathogen Occurrence in Irrigation Waters

JUSTIN FALARDEAU[1], Roger Johnson[2] and Siyun Wang[1]

[1]University of British Columbia, Vancouver, BC, Canada, [2]National Microbiology Laboratory at Guelph, Guelph, ON, Canada

Introduction: Current methods for assessing quality of irrigation water in Canada and the United States are based on the hygiene indicators, fecal coliforms (FC) and generic *Escherichia coli* (EC); however, debate exists as to whether these indicators are predictive of pathogen occurrence.

Purpose: Our objective was to compare the results from two different FC/EC enumeration methods and determine their ability to predict the presence of verotoxigenic *E. coli* (VTEC), *Salmonella*, and *Listeria monocytogenes* in irrigation water.

Methods: Water was collected bi-monthly over a 15-month period from seven irrigation ditches within two distinct watersheds in British Columbia, Canada. FC and EC were enumerated by 1) a 1 ml aliquot on 3M™ Petrifilm™ *E. coli*/Coliform count plates, and 2) a 25 ml membrane filtration with growth on m-FC agar with 0.01% rosalic acid, followed by transfer to nutrient agar containing 4-methylumbelliferyl-β-D-glucuronide. The samples were tested for the presence of VTEC using a verotoxin colony immunoblot (VT-IB), and for *Salmonella* and *L. monocytogenes* using Health Canada methods.

Results: The two methods were found to be significantly correlated for observed counts of both FC ($r = 0.60$; $P < 0.001$) and EC ($r = 0.77$; $P < 0.001$); however, the Petrifilm™ method gave higher numbers in general for both indicators. Enumeration by membrane filtration significantly correlated with pathogen presence for both FC ($r = 0.431$; $P < 0.001$) and EC ($r = 0.408$; $P < 0.001$), but the Petrifilm™ method only correlated significantly for FC ($r = 0.234$; $P < 0.001$). Regression tree analysis showed a split of an 83.3% chance of pathogen occurrence in samples with ≥ 7.54 CFU/ml FC when measured by membrane filtration.

Poster

Significance: This research shows that while FC and EC may correlate with the presence of pathogens in water, the enumeration method and volume tested may play a role in their accuracy.

P3-218 Generic *E. coli* Levels in Surface and Non-traditional Irrigation Water in the Mid-Atlantic in Relation to FSMA Water Quality Standards: A CONSERVE Study

SARAH ALLARD[1], Sultana Solaiman[2], Mary Theresa Callahan[2], Eric Handy[3], Cheryl East[3], Hillary Craddock Kelbick[1], Rianna Murray[1], Anthony Bui[1], Joseph Haymaker[4], Derek Foust[4], Samantha Gartley[5], Adam Vanore[5], Salina Parveen[4], Fawzy Hashem[4], Maryam Taabodi[4], Eric May[4], Kalmia Kniel[5], Manan Sharma[3], Shirley Micallef[2] and Amy Sapkota[1]

[1]Maryland Institute for Applied Environmental Health, University of Maryland, School of Public Health, College Park, MD, [2]University of Maryland, College Park, MD, [3]U.S. Department of Agriculture–ARS, Beltsville, MD, [4]University of Maryland Eastern Shore, Princess Anne, MD, [5]University of Delaware, Newark, DE

Introduction: The use of surface (pond and river) and nontraditional (reclaimed wastewater, produce wash water) irrigation water (SNIW) could reduce demand on groundwater resources. However, it is essential to understand how these irrigation sources may influence the microbiological safety of fresh produce, and how they comply with FSMA irrigation water quality standards (Geometric Mean (GM) of 126 CFU/100ml and Statistical Threshold Value (STV) of 410 CFU/100ml of *Escherichia coli*).

Purpose: To evaluate SNIW sources for the presence of generic *E. coli* and compare the GM and STV from these sites to FSMA standards.

Methods: Surface and nontraditional irrigation water (100ml) from 12 sites (*n*=51) in the Mid-Atlantic was collected and filtered for enumeration of *E. coli* by standard membrane filtration (EPA Method 1604). Water from 8 surface water sites, 3 reclaimed wastewater sites, and a produce processing facility were evaluated on up to 6 different dates (Sept–Dec 2016). *E. coli* was quantified on MI agar, and GM and STV were calculated from all available data.

Results: *E. coli* levels below 126 CFU/100ml were found in 47% of water samples analyzed. Of the 12 sites analyzed, 7 had a GM above the 126 CFU/100ml FSMA threshold. The FSMA STV of 410 CFU *E. coli* /100ml was exceeded in 10/12 sites examined. Of all surface and nontraditional water sources tested, only 2 sites, both reclaimed wastewater, had *E. coli* levels below GM and STV thresholds. Overall, samples collected from river water and vegetable processing sites contained higher levels of *E. coli*, while reclaimed wastewater (chlorinated before sampling) and pond water contained lower levels of *E. coli.*

Significance: Many surface and nontraditional irrigation water sources in the Mid-Atlantic analyzed from Sept–Dec 2016 would require mitigation before use as irrigation water for fresh produce crops to comply with FSMA standards.

P3-219 Die-off Rates of Surrogate and Virulent EHEC-STEC Strains from the Surface of Strawberry Plants Vary with Time, Inoculum Dose and Chemical Interventions

Maria Albarracin, Christopher Gunter, Siddhartha Thakur and **EDUARDO GUTIERREZ-RODRIGUEZ**

North Carolina State University, Raleigh, NC

Introduction: Agricultural water in contact with fresh produce during preharvest or postharvest operations may pose a risk to the microbial safety of produce. Current alternative provisions within FSMA allow bacterial in-field die-off rate of 0.5 log CFU/day between the last irrigation and harvest when water fails to meet microbial quality standards.

Purpose: Determine validity of die-off rate of 0.5 log CFU/day between the last irrigation event and harvest in NC strawberry production system and field environment.

Methods: Strawberry plants were grown following standard commercial practices under BSL2 greenhouse conditions. Generic *E. coli-W778*, Enterohemorrhagic *E. coli* (EHEC) and Shiga Toxin-Producing *E. coli* (STEC) strains were spot (100ul) inoculated to the surface of strawberry at log 6.0 and 3.0 CFU/ml. Bacterial recovery (4 replicates) was performed at 0, 2, 4 and 8 days post-inoculation (DPI); with or without chlorine or peroxyacetic acid (PAA) applications after 4-DPI at 100 and 60ppm concentrations, respectively. Environmental data was used for modeling die-off rates for each strain.

Results: EHEC, STEC and *E. coli-W778* die-off rates were strain, time and concentration dependent. However, all strains displayed bimodal die-off dynamics, with higher rates between 0 and 2-DPI and lower rates thereafter. Three out of the five EHEC-STEC strains inoculated at log 6.0 CFU/ml displayed die-off rates <0.5 log CFU/day throughout the experiment. At log 3.0 CFU/ml, average die-off rates were 0.31, 0.27 and 0.02 log CFU/day at 2, 4 and 8-DPI respectively. Similar die-off patterns were observed for *E. coli-W778*. Chlorine and PAA treatments marginally reduced the population (25%) of EHEC and STEC when inoculated at log 6.0 CFU/ml while at log 3.0 CFU/ml no conclusive effects were observed between strains.

Significance: Irrespective of pathogen fitness and virulence, die-off rates over 8-DPI did not adjust to a linear model as proposed by the FSMA Produce Rule.

P3-220 Screening of Non-traditional Irrigation Water Sources for Shiga Toxin-producing *Escherichia coli* in the Mid-Atlantic Region of the United States: A Conserve Study

JOSEPH HAYMAKER[1], Fawzy Hashem[1], Salina Parveen[1], Eric May[1], Manan Sharma[2], Chanelle White[1], Shirley Micallef[3], Maryam Taabodi[1] and Amy Sapkota[4]

[1]University of Maryland Eastern Shore, Princess Anne, MD, [2]U.S. Department of Agriculture–ARS, Beltsville, MD, [3]University of Maryland, College Park, MD, [4]Maryland Institute for Applied Environmental Health, University of Maryland, School of Public Health, College Park, MD

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Introduction: Nontraditional irrigation water sources (NTIWS) have become a national priority with regard to agricultural water security. The NTIWS that are being analyzed for potential use on fresh produce are tidal and non-tidal surface water and reclaimed water. As a result of the Food Safety Modernization Act, agricultural producers must meet stricter guidelines regarding the quality of irrigation water used on fresh produce.

Purpose: To evaluate the NTIWS for their physicochemical qualities and presence of Shiga toxin-producing *Escherichia coli* (STEC).

Methods: The physicochemical qualities (temperature, dissolved O_2, conductivity, pH, turbidity) were measured using an YSI EXO2 Multi-Parameter Water Quality Sonde. We developed and implemented a sampling apparatus which was used to filter the water samples through a modified cheesecloth membrane filter. Sixty samples were collected periodically in fall 2016 from six sites; 48 and 12 samples from four surface water sites (one tidal and three non-tidal) and two reclaimed water sites, respectively. Cheesecloth filters were enriched using universal pre-enrichment buffer followed by secondary enrichment in Non-O157 STEC enrichment broth. Samples were streaked on Non-O157 STEC chromogenic plating medium and CHROMagar-O157. Presumptive STEC colonies were confirmed using an 11-plex traditional PCR for serotypes (O26, O45, O103, O111, O121, O145) and stx_1, stx_2, *eae, ehx,* and O157:H7.

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Results: The sampling apparatus was successful and greatly enhanced sampling efficiency. One non-tidal site had two samples (~17%) positive for O45 and stx_2 gene, while another non-tidal site had one positive sample (~8%) for stx_1 gene. No samples were positive for O157:H7. No tidal or reclaimed water sites were positive for STEC.

Significance: The sampling apparatus has the potential of greatly improving the sampling process of surface and reclaimed waters for bacterial detection. These results indicate low prevalence of STEC in NTIWS. Sampling is ongoing and will continue for an additional three years.

P3-221 Evaluation of *E. coli* and Other Indicators as Predictors of Foodborne Pathogens in Irrigation Water

AMY KAHLER[1], Candace Miller[2], Mia Mattioli[1], Moukaram Tertuliano[3], Karen Levy[4], George Vellidis[3] and Vincent Hill[1]

[1]Centers for Disease Control and Prevention, Division of Foodborne, Waterborne and Environmental Diseases, Atlanta, GA, [2]ORISE, Atlanta, GA, [3]University of Georgia, Tifton, GA, [4]Emory University, Atlanta, GA

Introduction: Currently, produce growers in the U.S. use the fecal indicator bacteria *Escherichia coli* to evaluate irrigation water quality, using standards developed for recreational water monitoring. Evidence is lacking to demonstrate whether the FSMA *E. coli*standards accurately reflect the presence of foodborne pathogens in irrigation water. Alternative indicators may provide growers with a better understanding of the microbial quality of water used in produce production.

Purpose: This study examined whether the current *E. coli* standards were associated with pathogen presence in irrigation water. We then evaluated the utility of *E. coli*, other traditional fecal indicator microorganisms, and molecular microbial source tracking (MST) markers as tools for predicting the presence of pathogens.

Methods: Irrigation water was collected from three ponds in a southern Georgia growing region over one year. Samples were analyzed for *Salmonella* and fecal indicators *E. coli*, enterococci, and male-specific bacteriophages. Samples were also analyzed for MST markers of fecal contamination including: human, avian, ruminant, pig, and chicken specific targets. Logistic and linear regression were conducted to compare detection rates and concentrations of *Salmonella* and indicators.

Results: *Salmonella* presence was not associated with *E. coli* concentrations above the USEPA recreational water geometric mean standard of 126 MPN/100 mL or the statistical threshold value of 410 MPN/100 mL. There were no associations between the presence of any indicator to *Salmonella* presence. However, *E. coli* and enterococci concentrations were positively associated with *Salmonella* presence. *E. coli*, enterococci, and bacteriophage concentrations were also positively correlated with *Salmonella* concentration. The MST markers were not associated with *Salmonella* presence or concentration.

Significance: Our results show that recreational water quality standards for *E. coli* were not predictive of *Salmonella* presence in the irrigation ponds studied in southern Georgia. Further research is needed to evaluate *E. coli* guideline values and consider alternative agricultural water quality indicators.

P3-222 Persistence of *Escherichia coli* on Field-grown Tomatoes Inoculated with Contaminated Water Spray

CHANELLE WHITE[1], Fawzy Hashem[1], Patricia Millner[2], Joseph Haymaker[1], Annette Kenney[1] and Lorna Graham[1]

[1]University of Maryland Eastern Shore, Princess Anne, MD, [2]U.S. Department of Agriculture–ARS, Beltsville, MD

❖ ***Developing Scientist Competitor***

Introduction: According to the Centers for Disease Control and Prevention (CDC), fresh produce attributed to 46% of all foodborne illnesses in the United States that occurred from 1998 to 2008. Contamination of fresh produce can arise during any of several points in the production process including irrigation. Water used for irrigation and agricultural spray has been identified in the FDA Produce Safety rule as a potential source of produce contamination.

Purpose: To investigate the survival of total coliforms and generic *Escherichia coli* (gEc) populations on field-grown tomatoes irrigated with contaminated water.

Methods: In fall of 2015 and summer of 2016, tomato seedlings (variety: BHN 602 VFFF Hybrid) were transplanted into the field in complete randomized block experiments with four treatments and three replications/treatment. At breaker stage where fruits were pink/red-ripe, plants were spray-inoculated 10-days before final harvest with non-chlorinated water containing fresh bovine manure to achieve four target cell population treatments of 0, 100, 1000, and 10000 gEc/100mL. On days 0 (pre- and post-inoculation), 1, 3, 5, 7, and 9, populations of total coliforms and gEc were determined from tomato fruits (n=15/plot).

Results: In fall of 2015, populations of total coliforms and gEc ranged from 2.58 to 4.95 and 0 to 3.14 CFU/g, respectively, for tomatoes at all stages of ripeness. In summer 2016, total coliforms and gEc populations ranged from 2.27 to 4.20 CFU/g and 0 to 1.48 CFU/g, respectively. Results from both years showed that inoculation dosage had no significant ($P < 0.05$) effect on survival of total coliforms and gEc on tomato fruits.

Significance: Results indicate that unspecified environmental factors can influence total coliforms and gEc survival on tomato fruits prior to harvest; these factors remain to be clarified.

P3-223 Diversity and Fitness of *Listeria* spp. Isolated from Two Watersheds in Nova Scotia, Canada

Amit Ross[1], Kara Neudorf[1] and **LISBETH TRUELSTRUP HANSEN**[2]

[1]Dalhousie University, Halifax, NS, Canada, [2]Technical University of Denmark, Kgs. Lyngby, Denmark

Introduction: There have been many studies on the ecology and fitness (i.e., the ability of an organism to survive and reproduce under various environmental conditions) of *L. monocytogenes* in an effort to better understand the transmission pathways and the factors that help *L. monocytogenes* persist in food processing facilities. However, there are limited studies comparing the fitness of other *Listeria* strains, and most are limited to a few species such as *L. welshimeri*, *L. innocua*, and *L. ivanovii*.

Purpose: The purpose of this study was to provide a better understanding of natural reservoirs of pathogenic and other *Listeria* species, as well as the factors which may contribute to their survival in food processing environments.

Methods: Colony PCR and Sanger sequencing targeting the 16S ribosomal RNA and *sigB* genes were used to identify 679 naturally occurring *Listeria* strains isolated from fresh water samples obtained from one urban and one rural watershed in Nova Scotia, Canada. For each of the detected *Listeria* species, the fitness of two isolates from each watershed was further evaluated through assays testing motility, biofilm formation (100% RH, 15°C), desiccation tolerance (23% RH, 15°C) and resistance of planktonic and sessile cells to benzalkonium chloride (BAC).

Results: *L. monocytogenes* (26% of the 679 strains), *L. innocua* (52%) and *L. seeligeri* (18%) were isolated from both watersheds. *L. welshimeri* (3%) and *L. fleischmannii* (1%) were only isolated from the rural watershed. *L. fleischmannii* and *L. innocua* formed significantly ($P < 0.05$) less biofilm than the

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other species. *L. monocytogenes* strains from the urban and rural watersheds were the most and least desiccation-resistant, respectively. Generally, the non-motile *L. fleischmannii* was the most susceptible to BAC.

Significance: This research provides insights into natural reservoirs of pathogenic and non-pathogenic *Listeria,* and the fitness that may help *Listeria* spp. persist in food processing plants.

P3-224 Evaluation of *Listeria monocytogenes* Survival and Infectivity in Non-traditional Agricultural Waters

SAMANTHA GARTLEY[1], Adam Vanore[1], Shani Craighead[1], Manan Sharma[2] and Kalmia Kniel[1]

[1]University of Delaware, Newark, DE, [2]U.S. Department of Agriculture–ARS, Beltsville, MD

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Introduction: *Listeria monocytogenes* (*Lm*) is an enteric bacterium that can be found in environmental reservoirs. Restricted water availability for agriculture has increased interest in surface and reuse water sources which could potentially transmit *Lm*.

Purpose: Persistence and infectivity of *Lm* recovered from brackish tidal (TW) and vegetable wash water (VW) were compared.

Methods: Water was collected from the Mid-Atlantic region at two sites in Fall 2016. *Lm* (environmental isolate from 2011 outbreak associated with cantaloupes) was inoculated into 10ml of collected water at 8.62 log CFU/ml, all in triplicate, which was held for ten days at 16°C to mimic irrigation water temperatures during harvest. *Lm* was recovered on Brilliance *Listeria* Agar (BLA) on days 0, 3, 5, 7, and 10. Cell culture infectivity with recovered *Lm* from day 7 was performed by inoculating 1ml of sample onto human ileocecal monolayers (HCT-8) for 30 min, washing with HBSS, treating with 10µg/mL gentamicin sulfate for 30 min, and incubating at 37°C for one hour after which infective *Lm* cells were recovered and enumerated on BLA. Statistical analysis was performed by one-way ANOVA and t-test.

Results: Initial populations of *Lm* were 8.62±0.32 log CFU/ml on day 0 across water types. By day 7, *Lm* populations had significantly ($P < 0.05$) declined to 6.44±0.41 log CFU/ml in VW while *Lm* populations in TW remained at 8.33 ±0.16 log CFU/ml at day 7. Infectivity assay data for day 7 samples showed recovery of 5.06±0.32 log CFU, 3.97±0.36 log CFU, and 3.36±0.95 log CFU from BPW, VW, and TW, respectively, from infected HCT-8 cells, the inverse of the total day 7 counts for the VW and TW samples.

Significance: *Lm* inoculated into brackish tidal and vegetable wash waters showed decreased viability and decreased infectivity as demonstrated in a cell culture assay.

P3-225 Removal of *Listeria monocytogenes* and *Salmonella* Typhimurium from Water Using a Filtration System with Surfactated Modified Zeolite

JOSE BRANDAO DELGADO[1], Ligia Fragallo[2] and Marlene Janes[1]

[1]Louisiana State University, Baton Rouge, LA, [2]Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil

❖ ***Developing Scientist Competitor***

Introduction: Water quality has become one of the principal's focus of Food Safety control with the Food Safety Modernization Act (FSMA). Some produce farmers use surface water for irrigation that could have a higher bacterial content than allowed by the rule. Therefore, the removal of bacterial from irrigation water at a low cost has become a priority.

Purpose: The purpose of this study was to develop a filtration system with surfactant-modified zeolite (SMZ) to remove *Listeria monocytgenes* and *Salmonella* Typhimurium from irrigation water.

Methods: The SMZ was produced by treating zeolite with a solution of hexadecyltrimethylammonium bromide (HDTMA-Br) at 0, 10, 20, 30% (w/w) and drying at 125°C for 30 minutes. The testing of the SMZ were done using a liter of inoculated water with *Listeria monocytogenes* and *Salmonella* Typhimurium, at a concentration of 5 log CFU/ml, which was passed through a column with 20 g of SMZ. The concentration were determinate by plating one ml before and after filtration in Modified Oxford Agar and Xylose Lysine Deoxycholate agar respectively. All analysis were based on three separate experiments with each mean + standard deviation being average of six determinations.

Results: The SMZ at concentrations of 10, 20, and 30% HDTMA-BR was able to remove 5 log CFU/ml of *Listeria monocytogenes* from the water. The control treatment SMZ 0% HDTMA-BR did not remove *Listeria monocytogenes* from the water. The SMZ at concentration of 0% and 10% HDTMA-BR did not remove *Salmonella* from the water. The SMZ at 20% and 30% HDTMA-BR was able to filter out 0.4±0.02 log CFU/ml and 1.2±0.10 log CFU/ml of *Salmonella* from the water, respectively.

Significance: The SMZ was more effective at removing *Listeria monocytogenes* than *Salmonella* Typhimurium from water.

P3-226 Correlation of *Salmonella* spp. to Generic *Escherichia coli* in Irrigation Water

MOHAMMED ALHEJAILI[1], Dorra Djebbi-Simmons[1], Achyut Adhikari[2] and Marlene Janes[1]

[1]Louisiana State University, Baton Rouge, LA, [2]Louisiana State University AgCenter, Baton Rouge, LA

Introduction: *Salmonella* is one of the most causative foodborne disease outbreaks in the U.S.A. *Salmonella* has been detected in surface water used in irrigation and can survive for weeks to years in water.

Purpose: The purpose of this study was to detect low levels of *Salmonella* spp. by comparing surfactant modified zeolite (SMZ) filtration with the Environmental Protection Agency (EPA) method 1200 and determine if there was a direct correlation between *Salmonella* and generic *Escherichia coli* in irrigation water.

Methods: Irrigation water was collected monthly from May to October, 2016. Water samples (300 ml) were filtered through 3 g of SMZ. After filtration the SMZ was mixed with 10 ml of BPW to detach organisms. The presence of *Salmonella* spp. was examined using standard plating techniques for isolation and latex agglutination and RT-PCR for confirmation. The EPA 1603 method was used to determine generic *E.coli* counts.

Results: *Salmonella* was detected in water samples during four months using the EPA 1200 method, whereas it was only detected one month with the SMZ method. Generic *E.coli* counts ranged from 1.89 to 2.75 log CFU/100 ml of water. During June and July, *Salmonella* was not detected in the irrigation water, but the generic *E. coli* counts were 2.47 and 2.75 log CFU/100 ml water, respectively. In August and September, *Salmonella* was detected in irrigation water and the generic *E. coli* counts were 1.99 and 1.89 log CFU/100 ml, respectively.

Significance: The EPA method detected *Salmonella* at lower levels than the SMZ method. This study found no correlation between generic *E.coli* and presence or absence of *Salmonella* in irrigation water.

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P3-227 Prevalence and Concentration of *Salmonella* in Agricultural Water Used in Pre-harvest Production on the Eastern Shore of Virginia

LAURA TRUITT, Rachel Pfuntner, Jacob McClaskey, Steve Rideout and Laura Strawn
Virginia Tech - Eastern Shore AREC, Painter, VA

❖ ***Developing Scientist Competitor***

Introduction: Several produce-associated outbreaks of salmonellosis have been linked to irrigation with contaminated water. Minimal data exist on the prevalence and concentration of *Salmonella* in agricultural surface water on the Eastern Shore of Virginia.

Purpose: This study evaluated the prevalence, concentration, and diversity of *Salmonella* in agricultural surface water used in pre-harvest production.

Methods: Twenty agricultural ponds were sampled during the 2015 and 2016 growing seasons. A total of 400 water samples (250 mL) were cultured for *Salmonella* using standard methods. One representative isolate from each *Salmonella*-positive water sample was serotyped by the National Veterinary Services Laboratories. In 2016, a three-by-three tube MPN was used to estimate the concentration of *Salmonella* in each sample. Additionally, environmental and meteorological factors were analyzed for their association with the detection of a *Salmonella*-positive water sample using logistic regression analysis.

Results: *Salmonella* was detected in 19% of water samples in each year (38/200; 2015 and 38/200; 2016). For 2016, the average concentration of *Salmonella* was 0.10 MPN/mL, with a standard deviation of 0.16 MPN/mL. The highest concentration of *Salmonella* was 0.93 MPN/mL (n=1); however, 79% (158/200) of samples were below the limit of detection (0.03 MPN/mL). Of the 38 isolates sent for serotyping in 2015, nine different serovars were identified including Newport, Javiana, Berta, Norwich, Saintpaul, Thompson, Infantis, Senftenberg, and Typhimurium. Interestingly, 50% of the serovars were *Salmonella* Newport (n=19). Furthermore, precipitation on the day of or prior to sampling and pond location significantly increased the likelihood of detecting *Salmonella* (P< 0.05).

Significance: Volume of water tested had an impact on the probability of detecting *Salmonella* in water samples. While the prevalence of *Salmonella* was high, the concentration was extremely low and may not pose a public health risk, especially if water is not applied to the harvestable portion of the crop.

P3-228 Seasonality, Diversity and Indicators of *Salmonella* Contamination of Environmental Surface Waters of the Virginia Eastern Shore

CHRISTINA M. FERREIRA, Elizabeth Reed, Amir Mokhtari, Yan Luo, Jie Zheng and Rebecca L. Bell
U.S. Food and Drug Administration, College Park, MD

Introduction: Environmental surveys along the Virginia Eastern Shore (VES) have identified *Salmonella* as a persistent inhabitant of environmental surface waters. Since surface waters may contact fresh produce, via irrigation or field flooding, good indicators for *Salmonella* are needed for easier prediction of possible contamination.

Purpose: This study examined potential correlations between *Salmonella* concentration in surface waters along the VES and other biological and/or physical indicators; to characterize seasonality in *Salmonella* levels recovered during a one-year sampling period; and to examine serotype diversity.

Methods: Water and sediment samples were collected from six surface water sites in VES and two in West Virginia for a one-year period (October 2014 - September 2015). Physical parameters of the water were evaluated *in situ*. Water samples were enumerated for *Salmonella*, using a MPN method, and coliforms/fecal coliforms using the IDEXX Colilert® system. Sediment samples were tested for the presence of *Salmonella*. MPN estimates were compared to the physical water parameters on a statistical basis. *Salmonella* isolates were sequenced, and serotypes determined by molecular serotyping or genome sequence. Genomic comparisons were conducted using the FDA/CFSAN SNP pipeline.

Results: Preliminary results showed moderate correlations between log-transformed values (log10 MPN/100ml) and water temperature (°C) (0.51 and 0.53, *Salmonella* and fecal coliforms, respectively) as well as conductivity (0.38 and 0.37, *Salmonella* and fecal coliforms, respectively). Also a moderate correlation between log-transformed values of *Salmonella* and fecal coliforms was detected (correlation coefficient of 0.55). Over 500 isolates, representing thirty-two unique serovars were obtained. The number and diversity varied with site and month. Genomic analysis revealed over 430,000 SNPs between these diverse strains. The resultant tree sorted the strains by serovar with no polyphyly observed.

Significance: The data gathered in this study will aid in the development of risk assessment models and new preventative controls strategies designed to reduce or eliminate preharvest contamination of fresh produce.

P3-229 Assessment of Indicator Bacteria and *Aeromonas* spp. in Surface and Non-traditional Irrigation Water: A Conserve Study

SULTANA SOLAIMAN[1], Mary Theresa Callahan[1], Sarah Allard[2], Eric Handy[3], Cheryl East[3], Eric May[4], Fawzy Hashem[4], Salina Parveen[4], Kalmia Kniel[5], Manan Sharma[3], Amy Sapkota[2] and Shirley Micallef[1]
[1]University of Maryland, College Park, MD, [2]Maryland Institute for Applied Environmental Health, University of Maryland, School of Public Health, College Park, MD, [3]U.S. Department of Agriculture–ARS, Beltsville, MD, [4]University of Maryland Eastern Shore, Princess Anne, MD, [5]University of Delaware, Newark, DE

Introduction: The use of surface and nontraditional irrigation water (SNIW) (pond, tidal and non-tidal river water, reclaimed wastewater) is one way to conserve groundwater. However, SNIW may serve as a reservoir and vehicle for under-recognized enteric pathogens, spreading localized contamination during fresh produce cultivation.

Purpose: To investigate the presence of *Aeromonas* spp., gram-negative bacteria commonly found in water environments that can cause gastrointestinal disease, and bacterial fecal indicators (total coliforms (TC), *Escherichia coli*, *Enterococcus* spp.) in SNIW in Maryland.

Methods: Water samples from 2 ponds, 2 tidal rivers, 4 non-tidal rivers/creeks and 3 reclaimed wastewater treatment plants were collected in Maryland over a period of four months. Bacteria were enumerated using a standard membrane filtration method on MI (*E. coli*/TC) (n=49), mEI (*Enterococcus*) (n=49) and ADA-V (*Aeromonas*spp.) (n=43) agars. Water temperature, pH and turbidity were measured using a ProDSS multi-parameter meter.

Results: All samples tested (43/43) were positive for *Aeromonas* spp. The prevalence of *E. coli*, TC, *Enterococcus* spp. and *Aeromonas* spp. in water samples ranged from undetectable-4.1, 3.4-5.9, undetectable-4.6 and 2.5-5.5 log CFU/100mL of water, respectively. Mean counts were 2.9, 4.7, 3.3 and 4.3 log CFU/100ml, respectively. Non-tidal fresh water samples harbored significantly higher levels of *E. coli* and enterococci compared to pond and reclaimed water (P<0.05). *Aeromonas* counts were significantly correlated with *E. coli,* TC and enterococci (R^2=0.42, 0.36 and 0.43; P<0.0001), respectively. *Aeromonas* population levels were also weakly positively correlated with water temperature (R^2=0.17; P <0.01) and turbidity (R^2=0.12; P <0.05).

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Significance: This study is part of a two-year sampling effort to characterize the microbiological quality of surface and nontraditional water sources (CONSERVE). Results show that SNIW harbors high levels of *Aeromonas* spp. that correlate with bacterial fecal indicators and physical parameters of water. Risks posed by under-recognized pathogens in fresh produce need to be assessed.

P3-230 Microbial Quality of Tail Water in the California Central Coast Salinas Valley

ANNE-LAURE MOYNE[1], Laura A. Murphy[2], Michael D. Cahn[2], Steven T. Koike[2] and Linda J. Harris[1]

[1]University of California-Davis, Davis, CA, [2]University of California Cooperative Extension—Monterey County, Salinas, CA

Introduction: Many farms in the Salinas Valley have infrastructure to collect and reuse run-off water (or 'tail water') but few studies have systematically assessed the quality of this potential resource.

Purpose: To characterize the physicochemical and microbiological properties of Salinas Valley tail water.

Methods: Six tail-water reservoirs, representative of those used in Salinas Valley vegetable production, were sampled monthly for 1 year. Physicochemical water characteristics, including turbidity, temperature, pH, dissolved oxygen, conductivity, and oxidation reduction potential, were measured each time. Samples were plated onto plate count agar and incubated at 37°C for 24 h to determine the aerobic plate count (APC). Coliform and generic *Escherichia coli* concentrations were determined with both the Colilert Quanti-Tray 2000 system and by filtration and plating on CHROMagar ECC.

Results: APC for the tail-water samples ranged from 2.43 to 5.74 log CFU/ml (mean: 3.83 log CFU/ml). Coliforms were detected at all time points and sites, with concentrations from 0.78 to 5.30 log CFU/100 ml or 0.30 to 5.19 log MPN/100 ml (mean: 3.91 log CFU/100 ml or 3.18 log MPN/100 ml). Forty eight of 60 (80 %) samples were positive (≥ 1 CFU/100 ml) for generic *E. coli*. *E. coli* and coliform levels were significantly lower with the MPN method than with the filtration method. Concentration of *E. coli*, coliforms, or aerobic plate counts did not correlate with any of the measured physicochemical parameters (Pearson's correlation coefficients were not significant). Reservoir location was the main factor that significantly influenced *E. coli* concentration; one site consistently yielded higher *E. coli* concentrations. The *E. coli* geometric mean for this site was 486 CFU/100 ml or 248 MPN/100 ml compared to <38 CFU/100 ml or 20 MPN/100 ml for the other five sites.

Significance: Characterization of the microbial quality of tail water is a critical step to make informed decisions on its reuse.

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