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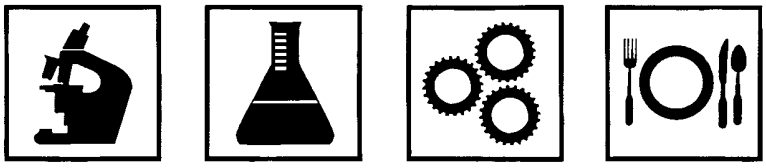

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ABSTRACTS

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IVAN PARKIN LECTURE ABSTRACT

Food Safety Advice for the Soul

Jeffrey M. Farber, Ph.D.
University of Guelph
Guelph, Ontario, Canada

A number of issues continue to pose significant challenges to global food safety. This includes, among other things, climate change, the emergence of new pathogens, an increasing population at-risk, consumer demands for a wider variety and fresher, more "natural" foods, and ingredients/foods being sourced from an increasingly greater number of countries. We need to do a better job of understanding how technology-driven food delivery will impact food safety. In relation to global food safety research needs, more funding should be allocated to areas such as food spoilage, novel foodborne viruses and mycotoxins. The safety of low-moisture foods and produce will continue to be strong areas of focus, while advances in food and host microbiome research will continue at an accelerating pace. Whole genome sequencing, including analyzing gene expression by using RNA sequencing technology, has already started to revolutionize the field of food safety and will continue to do so. Food safety regulations, which are becoming more outcome-based, need to keep pace with the latest advances in science. We have huge challenges in the risk communication area, as governments and companies often struggle to get ahead of the curve and come out on top in the social media trenches. Small and medium-sized businesses need help in understanding new emerging technologies and in coping with new regulatory requirements.

The consumer education area is still fragmented and not well-organized in many countries. We need to focus more on initiating food safety education at the primary school level. With regards to university level education, more needs to be done to develop global common curricula and learning outcomes for food safety degrees, including programs in food safety leadership. Students need to be given practical advice and should be taught the soft skills that they will need to get ahead in the workplace. Globally, we need to do more to teach and promote the basic tenets of One Health, which encourages an interdisciplinary and integrated approach, and which promotes a multi-sectoral and collaborative strategy focused on understanding and preventing risks at the interface between humans, animals and their environment.

Although issues still remain and will continue to challenge us, we have made great strides in many areas of food safety. We can and will continue to make progress, by having all those involved in the global safety of the food chain working together more closely in the food safety space in a non-competitive manner. As food trade expands throughout the world, food safety has become a mutual concern among both developed and developing countries, and we need to recognize that globally, we should do more to help disadvantaged countries develop robust food safety control systems.

JOHN H. SILLIKER LECTURE ABSTRACT

Improving Food Safety Globally: Developing and Applying Science for the Common Good

Renata Clarke, Ph.D.
Food and Agriculture Organization
of the United Nations

Local realities vary greatly with respect to the conditions under which food is produced, procured and consumed. At the same time, supply chains and markets keep us inter-connected. We have to be concerned with capacities of all countries to assure that food is reliably produced safety within their territories. For countries with "mature" systems of food control, it is a smart investment to help less advanced countries build scientific and technical capacities that support identification and management of food safety risks.

In many developing countries, particularly low income and medium-low income countries, the implementation of food control remains weak, despite the fact that many of them have been participating regularly within the Codex system for the last 15 years. Over this period, Codex has developed numerous science-based Codes of Practice and Guidelines aimed at promoting risk-based control. These codes require interpretation and adaptation to each context. This can be particularly challenging for many developing countries given the difficult and complex conditions under which food businesses often operate. More emphasis needs to be placed on how countries are able to take up Codex guidance.

The Codex SP 2014-2019 recognizes, among its strategic goals, the importance of increased scientific input from developing countries into the Codex processes. There has been, up to now, relatively little provision of data in response to FAO/WHO calls for data to support the development of scientific advice that guides the decisions of the Commission. This is one of the reasons for which a 2010 review of developing countries' participation in Codex concluded that they were increasingly involved in decision-making but less engaged in decision-shaping.

There have been a few occasions where projects have been implemented to assist developing countries to generate data where these were considered essential to inform standard development. Frequently, however, this is not possible and there can be no response to requests from developing countries for standards that they consider to be of importance particularly for their market access due to data gaps.

There are a number of emerging global food safety and "One Health" issues that can only be better understood and controlled if we have global data. There are problems of emerging zoonosis, anti-microbial resistance (AMR) and a number of climate-change related phenomena that are impacting significantly on food safety. The development of rapid, low cost and validated diagnostic methods could be of great value in enabling broader contribution to global intelligence. Other innovations that could be applied along food chains, such as water-clean-up technologies, would also be of value in promoting safe food production. We need to be more systematic in identifying innovations that could significantly improve food safety management in least developed countries and promoting work on these in research centres.

One recent food safety innovation that has been developed and applied in some African countries is biocontrol of aflatoxin during primary production of maize and peanuts. There have been positive reports on the efficacy of this technology. If this is verified, it will have a major impact on public health and food security. It will be important not to lose sight of the fact that fumonisins are also of major concern in maize.

Many developing countries have established programs to develop GM applications that can improve productivity and respond to challenges of climate change. It is important that donors not only support application development but also support national capacities to carry out risk assessment in accordance with existing Codex Guidelines. With increasing numbers of countries engaging in GM development on a widening range of commodities, it seems likely that inadvertent events of Low Level Presence of GM material in traded commodities are likely occur with increasing frequency. In the absence of harmonized approaches to risk management of such events, there needs to be better understanding on what the impact of resulting trade disruptions would be.

Food safety is at the heart of public health, economic and social development agendas. The scientific and academic communities have a major role to play in enabling transfer of knowledge and "know how" to improve food safety across the globe. They must also help us to understand the new food safety challenges and provide the evidence that enables sound, just and courageous policy.

Special Symposium Abstracts

SS1 Fresh, Local...and Safe: Supply Chain Food Safety Challenges in Meeting Consumer Trends

MANPREET SINGH: *Purdue University, West Lafayette, IN, USA*
WILL DANIELS: *Will Daniels Consulting, Carmel, CA, USA*
CHARLES SEAMAN: *Hy-Vee, West Des Moines, IA, USA*
MANSOUR SAMADPOUR: *IEH Laboratories & Consulting Group, Lake Forest Park, WA, USA*

There has been a rapidly growing consumer interest in food and beverage products and restaurant menu items featuring local ingredients. Consumers consider local and/or fresh ingredients in products to be associated with a higher quality product that supports small business and entrepreneurs with 52% of consumers stating that they view local as more important than organic in their purchasing decision. Aside from marketing appeal, food quality and safety concerns, transportation costs and local economic development efforts are compelling restaurants, groceries, and wholesalers to engage smaller producers and localize their supply chains. In turn, they are nurturing a "fresh food fast" procurement model that is growing from the ground up. The "source local" trend stems from the perception that the globalized food supply system involves importation of too many foods with little food safety oversight. However, even with the impetus to source local ingredients, there is an information gap on the challenges and risks associated within the diverse and complex commodity chain. Traceability, thorough food safety practices, and verification activities are some of the missing elements in many local and regional supply chains.

SS2 The Flint Water Crisis – What Happened and Lessons Learned

JOYCE ZHU: *Virginia Tech, Blacksburg, VA, USA*
KEVIN BESEY: *State of Michigan, Michigan Department of Agriculture & Rural Development, Lansing, MI, USA*
STAN HAZAN: *NSF, Ann Arbor, MI, USA*

In order to reduce costs, the city of Flint, MI in April 2014 switched their source of municipal water from water treated by the city of Detroit to untreated water sourced from the Flint river. Complaints from residents of poor water color, taste and odor began immediately after the switch. While authorities communicated this was a quality issue related to the change in water source it was determined that elevated blood lead levels in infants and children nearly doubled following the switch to the Flint river. After confirming elevated lead levels in water tested from homes of Flint residents it was determined orthophosate was not added to treat the flint river water. The river water was more caustic, containing a high concentration of chloride, which corroded the pipes resulting in leaching of lead into the drinking water. In January, 2016 the Governor of the State of Michigan declared the Flint Country (Genesee) to be in a state of emergency. This symposium will hear from a researcher from the Virginia Tech laboratory that has been central to discovering the lead contamination as well as the perspective from State of Michigan. We'll also hear about the risk of lead contamination due to our national aging infrastructure

SS3 An Update on Microbiological Testing in Food Safety Management

ROBERT BUCHANAN: *University of Maryland, College Park, MD, USA*
KATHERINE MJ SWANSON: *KMJ Swanson Food Safety, Inc., Mendota Heights, MN, USA*
MARCEL ZWIETERING: *Wageningen University, Wageningen, Netherlands*

This session is intended to provide an update on microbiological testing as part of managing and controlling food safety, focusing on the latest concepts and guidance developed by the International Commission on Microbiological Specifications for Foods (ICMSF). Already in 1974, ICMSF put forth a sound statistical basis for useful microbiological testing, establishing the concepts of sampling plans and microbiological criteria for foods in international trade, which have been adopted by Codex Alimentarius and others. Nowadays, microbiological testing is applied to food safety and quality management in a number of ways. Governments may use pathogen or indicator testing for lot inspection or verification as a means of lot acceptance, for example at port of entry or for surveillance activities on products in commerce. Industry may use end-product tests for lot acceptance in customer-supplier relationships, while microbiological testing may also be used to design safe and stable products, or to verify the adequate performance of particular processes or control measures for food safety/spoilage or of the overall food safety management system (i.e. HACCP and pre-requisite programs; FSMA preventative controls based safety plan). When using microbiological tests to evaluate safety or quality of food, it is important to select and apply these with knowledge of their limitations, their benefits and the purposes for which they are intended. This session therefore will provide an update on the circumstances in which microbiological testing may play a useful role in the frame of modern, risk-based food safety management approaches, practical examples of such useful testing for a variety of foods and processing environments, and the latest concepts and tools supporting the statistical basis and operation of sampling plans and microbiological criteria.

Symposium Abstracts

S1 Allergen Control in Food Facilities

JENNY SCOTT: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
DAVID BLOMQUIST: *Ecolab Inc., St. Paul, MN, USA*
LAUREN JACKSON: *U.S. Food and Drug Administration-CFSAN, Bedford Park, IL, USA*

Allergens represent a serious and potentially life-threatening food safety risk. According to the FDA millions of Americans have allergic reactions to food and food ingredients every year and controlling this problem is a major concern for all parts of the food industry. For food processors, incorrect allergen warnings represent the most frequent cause of food recalls. For retailers and food service, allergens represent a very real, immediate, and growing threat. The prevalence of food allergies among children increased 18% during 1997–2007, and allergic reactions to foods have become the most common cause of anaphylaxis in community health settings. In 2006, about 88% of schools had one or more students with a food allergy. Although there is a good understanding of the risk of allergens, the ways to control that risk is less clear. The only way for a person who has a food allergy to control their risk is complete avoidance of the food(s) they are allergic too. The question then for food processors, retailers, and food service operations is: How can we help those with food allergies avoid the items they are allergic to? This symposium will discuss allergen control and risk mitigation in a variety of food processing and handling settings. It will address sanitation solutions that do, and sometimes do not work for allergen control. How to validate those control measures and the impact that FSMA and other regulations have on allergen control.

S2 Allergen Management and Control in Retail and Food Service

ASHLEY EISENBEISER: *Food Marketing Institute, Arlington, VA, USA*
HAL KING: *Public Health Innovations, Atlanta, GA, USA*
DAN FONE: *NSF International, Ann Arbor, MI, USA*
ANTHONY LUPO: *Neogen Corporation, Lansing, MI, USA*

Food allergens present a significant health hazard to millions of consumers around the world. Symptoms can range from mild illness to anaphylactic shock and death. Globally, it has been estimated that about 240 – 550 million people may suffer from food allergy, with up to 15 million in the United States alone. The prevalence of food allergy is greater among children and have increased as much as 50% in the last two decades. As food producers struggle to comply with labeling laws, mislabeled and undeclared allergens remain one of the most common reasons for food recalls. The Food Allergen Labeling and Consumer Protection Act (FALCPA) was enacted in 2004 to enable the FDA to regulate both locally produced and imported food labels, and further strengthened in 2011 under the United States Food Safety Modernization Act (FSMA) with a provision for a preventive food allergen control program. While the United States recognizes 8 major food allergens, other countries may recognize up to 16 major allergens creating, even more, confusion for both producers and consumers. This symposium will feature industry experts who will outline the challenges and solutions from the retail, restaurant, consulting and laboratory perspectives. The speakers will discuss food production strategies on how to manage allergen risk, how to conduct efficient and effective recalls, and training for food workers in an industry with high turnover, controlling allergen risk from an international perspective, and the benefits and drawbacks of laboratory testing.

We are aware that the Hygiene and Sanitation PDG is also proposing an Allergen Symposium; from a cleaning and verification perspective. While we believe that the two symposia will work out well in tandem, we are also open to merging them into a single full symposium.

S3 The Complexity of Antibiotic Resistance – The Need for Multi-system Approaches

GABRIEL PERRON: *Bard College, Annandale-On-Hudson, NY, USA*
HEATHER HARBOTTLE: *U.S. Food and Drug Administration/Office of New Animal Drug Evaluation, CVM, Rockville, MD, USA*
TIMOTHY LAPARA: *University of Minnesota, Minneapolis, MN, USA*
SOPHIA KATHARIOU: *North Carolina State University, Raleigh, NC, USA*
JULIAN COX: *The University of New South Wales, Sydney, Australia*

Antibiotic resistance is an important subject with serious public health implications. Microorganisms have an arsenal of complex physiological mechanisms to become resistant to different antibiotics, even under the most regulated use of these compounds. Not surprisingly, antibiotic resistance was found soon after the discovery of antibiotics themselves. Current levels of antibiotic resistance pose a threat to the efficacy of these important therapeutic agents. The complexity of this problem demands concerted, international mitigation efforts associated with strong surveillance to monitor progress. We proposed a hybrid half symposium/half roundtable organized by representatives of multiple PDGs to provide an overview of the current understanding of key areas within this complex topic. Participants will understand the 1) Goals of the National Action Plan for Combating Antibacterial-resistant Bacteria; 2) Antimicrobial resistance in environmental bacteria; 3) Current knowledge on the impact of different production systems; 4) Use of antibiotic in other less-regulated industries; 4) Need for combined efforts to successfully control antibiotic resistance.

The symposium (60 minutes, three 20-minute talks) will include:

- Gabriel G. Perron, Bard College. Monitoring antimicrobial resistance in environments
- Heather Harbottle, Food and Drug Administration. Lessons learned since 2003: FDA's pre-approval microbial food safety approach to antimicrobial drugs used in food-producing animals
- Timothy LaPara, University of Minnesota. Use of antibiotic in other less-regulated industries
- The roundtable (30 minutes) will include:
- Sophia Kathariou, North Carolina State University. Impact of different food production systems on antibiotic resistance
- Julian Cox, University of New South Wales. Use of antibiotics by the animal industry in Australia

S4 Antimicrobial Resistance: The Ever-expanding Global Concern

MEGAN JACOB: *North Carolina State University, Raleigh, NC, USA*
AWA AIDARA-KANE: *World Health Organization, Geneva, Switzerland*

Antimicrobial resistance (AR), and in particular multi-drug resistance (MDR), is increasing in many different microorganisms recovered from a wide variety of sources including the environment, humans and animals. Global dissemination and zoonotic transmission of resistance genes and bacteria occur regularly. The environment is increasingly recognized as a major player in the AR story and the ecological importance of microbial communities

and the resistance attributes therein are an exciting area of study. The diagnostic laboratory, particularly the veterinary diagnostic laboratory, provides this new area of study and is also critically important as new and emerging diseases, resistance attributes, resistant gene combinations, and other confounding attributes are discovered. Additionally, AR is the focus of the World Health Assembly and many countries are adopting action plans on the issue; AR surveillance systems are also being implemented. In 2008 the WHO established the Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) to support their efforts to minimize the public health impact of AR associated with the use of antimicrobials in food animals. Over 30 international experts comprise the group who support WHO and other countries in implementing the Global Action Plan on Antimicrobial Resistance (GAP).

http://apps.who.int/gb/ebwha/pdf_files/WHA68/A68_20-en.pdf?ua=1).

Finally, the use of whole genome sequencing (WGS) has added a new dynamic to the field with respect to identification of resistance genes and use in surveillance. The challenges and benefits of using WGS in routine surveillance will be discussed.

This symposium presents new program information including the latest advances/knowledge on AR from an ecological perspective involving the veterinary diagnostic laboratory followed by international perspectives on surveillance systems including an overview of AGISAR from the WHO. The symposium will conclude with the challenges and benefits associated with implementing whole genome sequencing for surveillance.

S5 Small Scale Fermentation at Retail, is the Consumer at Risk?

KEVIN SMITH: *U.S. Food and Drug Administration, College Park, MD, USA*
FRED BREIDT: *U.S. Department of Agriculture-ARS, Raleigh, NC, USA*
DEOG-HWAN OH: *Kangwon National University, Chuncheon, South Korea*
MARISA BUNNING: *Colorado State University Extension, Fort Collins, CO, USA*
BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*

Fermented vegetable products are exploding (sometimes literally!) in popularity across the United States. Products range from imported 'traditional' fermentations to novel 'craft' fermented vegetables being prepared and/or sold at farmers markets, restaurants, grocery stores, and other retail establishments. For many small producers, retail operators, consumers, and regulators, key questions about the safety of these products remain unanswered. This symposium will, therefore, address questions about what constitutes a properly (safely) fermented product. The discussion will focus on questions about: are some products more hazardous than others? Is botulism a concern? What is the role of salt, pH and bacterial competition in fermentation safety? What factors should be considered as critical limits or controls for fermentations such as kimchi, kombucha, tempeh and others? Attendees will learn about the variety of products marketed today from a research, regulatory, and retail food safety perspective.

S6 Retail Food Safety Risks: Mobile Food Trucks, Grocery Stores, Raw Fish Preparation Practices, and Slicer Cleaning and Inspection Practices

JOYCE TUTTLE: *California Department of Public Health, Sacramento, CA, USA*
NICOLE HEDEEN: *Minnesota Department of Health, St. Paul, MN, USA*
DANNY RIPLEY: *Metro Nashville Public Health Department, Nashville, TN, USA*
LAUREN LIPCSEI: *CDC, Atlanta, GA, USA*

Half of all foodborne illness outbreaks are associated with retail establishments (restaurants and delis). To better understand the environmental causes of retail-related foodborne illness outbreaks, and subsequently reduce or mitigate them, the Centers for Disease Control and Prevention's (CDC) Environmental Health Specialists Network (EHS-Net) conducts studies on food preparation practices and other factors that could contribute to foodborne illness outbreaks. Speakers at this symposium will present results from four such studies and discuss them within the framework of improving food safety practices.

S7 Vomiting in Norovirus Transmission: Risk of Food Contamination?

LEE-ANN JAYKUS: *North Carolina State University, Raleigh, NC, USA*
AMIR MOKHTARI: *RTI International, Research Triangle Park, NC, USA*
HAL KING: *Public Health Innovations, Fayetteville, GA, USA*

Human noroviruses are responsible for the majority of outbreaks of acute viral gastroenteritis worldwide and are a leading cause of foodborne illness. Fecal material from infected individuals plays a key role in norovirus transmission. However, vomiting is the hallmark symptom of illness, and virus particles are present in human vomit, perhaps at very high concentrations. Epidemiological evidence has long supported the hypothesis that aerosolization of norovirus during projectile vomiting contributes to transmission, including the contamination of food. However, recent laboratory evidence supports this and actually provides quantitative data of norovirus aerosolization that can be used in support of risk modeling. The purpose of this short symposium is to examine the significance of vomiting in foodborne norovirus transmission, and what is being done to contain this potential contamination source.

S8 New Perspectives on Norovirus

ROBERT ATMAR: *Baylor College of Medicine, Houston, TX, USA*
MARTIN D'AGOSTINO: *Campden BRI Group, Chipping Campden, United Kingdom*
MARGARET HARDIN: *IEH Laboratories and Consulting Group, Lake Forest Park, WA, USA*

Norovirus, the number one cause of foodborne illness in the U.S., still remains a dynamic problem for the food industry and the scientific community. This symposium will emphasize significant progress and current knowledge gaps from a wide range of topics including diagnostics and biology to further our understanding and enhance our ability to control this virus. More specifically, the perspectives for, and against, standardization, as this has been applied to food testing protocols, will be evaluated. In addition, revelations identified from the soon to be published USDA/FDA National Advisory Committee on Microbiological Criteria for Foods report on norovirus will be highlighted that will provide useful insight to help steer future research priorities. Lastly, as recent achievements to reproduce virus replication have had some, albeit limited success, several research labs may be on the cusp of developing a viable *in vitro* culture system for this virus. A detailed state to the art presentation on norovirus replication efforts describing how close we may be to a viable *in vitro* testing method will be also presented.

S9 Decoding the Exchange between Human Pathogens and Plants: Attachment, Metabolism and Recognition

SHIRLEY A. MICALLEF: *University of Maryland, College Park, MD, USA*
JERI BARAK: *University of Wisconsin-Madison, Madison, WI, USA*
KALI KNIEL: *University of Delaware, Newark, DE, USA*
MAELI MELOTTO: *University of California-Davis, Davis, CA, USA*

Although plants are not the main reservoir for enteric bacteria and viruses, consumption of produce is the leading cause of foodborne illness in the United States. Our understanding of how these pathogens can associate with, and persist on plants to cause disease remains fragmented. For these associations to be successful, enteric bacteria must attach to plant surfaces, and avert recognition and/or thwart plant defenses, while competing with other microorganisms to access utilizable nutrients to sustain their growth and persistence. All this is done through a chemical exchange that we are just beginning to decode. Recent advances in research on human pathogen-plant interactions are revealing bacterial nutrient sources in the phyllosphere of food crops. Ongoing studies are investigating how human pathogens are able to thrive on plant surface compounds and exudates in a plant cultivar-dependent manner, revealing a controlled association between the bacterium and the plant genotype. Moreover, plant pathogens that cause plant cell damage and leakage of phytocompounds alter the phyllosphere niche in a way that may favor human pathogen colonization. Plants, on the other hand, appear to recognize human pathogens, mounting weak responses against the colonizers. Research on viral strategies is revealing how these infectious agents can persist on crops by binding to carbohydrates and proteins in a process that appear to vary by plant developmental stage. This symposium will bring together speakers who will present and discuss our current understanding of how enteric pathogens attach, persist and multiply on plants, while in turn being sensed by plants via pathogen-associated molecular patterns. As we continue to decode these interactions at the chemical level, we can begin to integrate this knowledge in future intervention strategies aimed at reducing contamination of fresh produce with enteric pathogens both at the pre- and post-harvest stage.

S10 On-farm Microbial Ecology: Addressing Complicated Interactions with Food Safety Implications

JAMES WELLS: *U.S. Department of Agriculture-ARS, Clay Center, NE, USA*
GANYU GU: *Virginia Tech, Painter, VA, USA*
SHIRLEY A. MICALLEF: *University of Maryland, College Park, MD, USA*

Metagenomics and transcriptomics can be useful tools to study the ecology of foodborne pathogens on the farm. They can help us understand the critical control points at the pre-harvest level, thereby aiding in the development and implementation of control measures. On-farm food pathogen ecology is an under-researched area and limitations due to the complex environmental matrices complicate it further. The symposium will discuss the use of molecular techniques, especially metagenomics and transcriptomics, to study microbial ecology and host-pathogen interactions at the pre-harvest level. The objective of this symposium is to discuss the use, applications, and limitations of current techniques commonly used to study molecular ecology as it relates to food safety.

S11 The Next Big Thing: Emerging Biological, Physical, Chemical and Cyber Threats to the Food System

SURESH D. PILLAI: *National Center for Electron Beam Research, College Station, TX, USA*
NATASHA HOCHBERG: *Boston University School of Medicine, Boston, MA, USA*
SHAUN KENNEDY: *University of Minnesota, St. Paul, MN, USA*
DANIEL SNOW: *University of Nebraska-Lincoln, Lincoln, NE, USA*
JASON WHITE: *Connecticut Agricultural Experiment Station, New Haven, CT, USA*
JESSICA PULZ: *U.S. Department of Agriculture - FSIS, Washington, D.C., USA*

The traditional canon of established threats to the safety of the food supply is daunting as is. However, a variety of societal, environmental and technological factors are driving the emergence of new and unprecedented threats. Globalization of the food supply and increased international travel has led to greater consumption of foods once considered "exotic", exposing new populations to viral, bacterial, protozoan or helminthic pathogens. Large and sustained outbreaks of communicable diseases, such as Ebola, not only impact food distribution and security, but may also create new opportunities for wider transmission through food vectors. Environmental contaminants such as steroids, antibiotics, pharmaceuticals or pesticides may find their way into human food systems via agricultural runoff, and the risks to the food supply and to human health of engineered nanomaterials are not yet fully understood. Finally, in an increasingly interconnected digital world, compromise of food formulation, refrigeration, distribution and related cyberinfrastructure could have severe food safety consequences. This session brings together a number of experts from various disciplines to discuss emerging threats to our food supply and how these threats can be addressed and contained.

S12 Harmonizing Hygiene and Sanitation Specifications for Improved Public Health and Better International Trade

PETER BEN EMBAREK: *WHO, Geneva, Switzerland*
CAROLINE SMITH DEWAAL: *U.S. Food and Drug Administration-CFSAN, Washington D.C., D.C., USA*
ANTHONY FLOOD: *International Food Information Council, Washington, D.C., USA*

The World Health Organization estimates that each year there are an estimated 2 million deaths worldwide from food and waterborne diseases, including many children. While foods and food processing equipment increasingly cross borders to reach foreign markets, implementation of preventive environmental food safety controls such as those in FSMA continues to be a challenge, especially in developing nations of the World with limited scientific and regulatory capacity. As food safety professionals we can save lives by working together globally to improve our methods of educating all countries on FSMA preventive controls from the farm to the fork. FSMA requires food safety measures that start at the grass root level and continue throughout the global supply chains to increase the harmonization of food safety specifications. Improved technical education can provide a deeper understanding of the hazards (microbiology, chemistry, etc.), preventive controls, documentation control and systems validation. The Food and Agriculture Organization of the United Nations is building capacity at the farm level; here Good Agricultural Practices and related farm systems must be translated and taught for compliance of FSMA. For example, FSMA stipulates addressing pathogens at the species and subspecies level. The International Food Information Council and United Nations Industrial Development Organization could be engaged to educate the global supply chain and accelerate sustainable adoption of FSMA standards at all levels. The symposium will present the current state from a global perspective, reveal differences in preventive control practices between countries and provide recommendations to better translate the needs for international adoption of the FSMA standards to improve public health and international trade.

S13 Alternative Solutions to Cleaning – Bringing Enzymatic and Other Cutting-edge Technologies to Successfully Managing *Listeria monocytogenes* in the Retail and Food Service World

THOMAS FORD: *Ecolab, Inc., Greensboro, NC, USA*
HALEY OLIVER: *Purdue University, West Lafayette, IN, USA*
ANNA STAROBIN: *Ecolab, Inc., Greensboro, NC, USA*
LARRY KOHL: *Food Lion Family – Delhaize America, Salisbury, NC, USA*

Listeria monocytogenes (*LM*) has proven to be present and persistent in the retail environment. The challenges in addressing the issue have proven that a multifaceted approach must be taken to address and have a meaningful impact on *Listeria's* ability to inhabit a retail environment. An approach that takes into account the unique features and characteristics of the bacteria, the unique operational and facility design of the retail store and an evolving and targeted combination of technology and procedures of a cleaning a sanitation program all must be a component of a successful *Listeria* management plan.

This session will involve three speakers that will present the latest scientific data about the scope and depth of the prevalence of *Listeria* at retail, the unique operational and facility challenges a retail environment presents and strategies that have proven successful in addressing the *Lm* issue. Additionally, a presentation of analytical data and learnings from an extensive field analysis conducted in 2015 of over 100 retail stores will be shared. The study showed remarkable success in identifying *Lm* locations, removing *Lm* from floors and drains, using specific tools and technologies and provided new insights about facility design and material substrates that can either complicate or enhance a food operator's success in managing *Listeria* in an environment.

S14 Tackling the Long-standing Challenge of *Salmonella* and Poultry with New Uses of Data and Partnerships

CRAIG KIEBLER: *Metabiota, San Francisco, CA, USA*
BOB O'CONNOR: *Foster Farms, Livingston, CA, USA*
JOHN LINVILLE: *U.S. Department of Agriculture - FSIS, Omaha, NE, USA*
FRANK YIANNAS: *Walmart, Bentonville, AR, USA*
ROBERT TAUXE: *Centers for Disease Control and Prevention, Atlanta, GA, USA*

Salmonella and poultry have been in a long-term relationship; that relationship is not a healthy one from the consumer's perspective. Food safety professionals are continuously identifying new ways to minimize consumer exposure to *Salmonella* and exploring methods to track our progress in preventing poultry-associated foodborne illness. This symposium looks at new uses of data and partnerships and additional efforts undertaken across the farm to table continuum by the U.S. food industry and federal public health agencies to prevent salmonellosis associated with consumption of poultry.

S15 Is *Salmonella* an Adulterant in Raw Meat and Poultry?

CARL CUSTER: *Retired, Bethesda, MD, USA*
JULIAN COX: *The University of New South Wales, Sydney, Australia*
DENIS STEARNS: *Marler Clark, Seattle, WA, USA*

This symposium will address the scientific, regulatory, and political issues surrounding whether *Salmonella* is or should be declared an adulterant in raw meat and poultry. One speaker will cover the epidemiology of salmonellosis, consumer handling, and the half century of scientific data addressing consumer's ability to safely handle raw muscle foods. The second speaker will review the diversity of the genus *Salmonella* including the pathogenicity of different strains to humans. The third speaker will discuss the law and court cases involving USDA, *Salmonella*, and consumers.

S16 Quantifying Bacterial Cross-contamination and Transfer: Importance in Risk Assessment

DONALD SCHAFFNER: *Rutgers University, New Brunswick, NJ, USA*
MAARTEN NAUTA: *DTU Food, Søborg, Denmark*
YUHUAN CHEN: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*

Bacterial transfer processes, including cross-contamination, can be important factors in food safety management. Contamination of products can take place by direct or indirect contact with other contaminated products, surfaces or the environment. Examples of transfer processes include contamination of food products from the processing environment; transfer from "food to surface to food" during cutting and slicing; redistribution of bacteria (e.g., during cutting and washing of vegetables), grinding and slicing of meat products; fecal contamination of produce during application of biological soil amendment or of meat during the slaughter process. The intricate nature of these processes complicates risk assessment and management, in industrial, retail and household setting. In some cases improved food hygiene may be a solution, in other cases, these transfer processes may also be an inevitable side effect of the food production and processing (e.g., defeathering of poultry or harvesting produce from the soil). Transfer studies and quantitative predictive models are essential to improve our understanding of cross-contamination and transfer and will lead to improved risk assessments and more effective food safety management. The number of studies on cross contamination is limited and predictive models for bacterial transfer are less well developed than models for bacterial growth and inactivation. An understanding of the best ways to link bacterial growth/inactivation models with bacterial transfer models is necessary to represent these distinct and often very different components in a risk assessment. In recent years, significant progress has been made in the development of these types of models, where they are supported by an increasing amount of data. This symposium aims to present the challenges and latest advances in this area, with the aim to illustrate their practical value and their integration into risk assessments.

S17 Environmental Monitoring: A New Approach to Norovirus Risk Management?

LEE-ANN JAYKUS: *North Carolina State University, Raleigh, NC, USA*
GEUN WOO PARK: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
INGEBORG BOXMAN: *Dutch Food and Consumer Product Safety Authority, Wageningen, Netherlands*
ANGELA FRASER: *Clemson University, Clemson, SC, USA*

Human noroviruses are the leading cause of acute gastroenteritis and the main cause of foodborne disease in the U.S. Among foodborne norovirus outbreaks, 81% occur in commercial food service settings and 3% in health-care settings. Most foods are contaminated at the point of preparation or service and infected food handlers play an important role in the transmission of the virus, either by fecally contaminated hands or surfaces; occasionally as a consequence of vomiting incidents. Identification of frequently touched surfaces and proper decontamination regimens are key measures to minimize food contamination in food service operations. Recently, surface sampling techniques have been refined so that we are now able to

quantitatively detect norovirus RNA on hard and even some soft surfaces. These methods facilitate characterization of environmental contamination with norovirus; investigation of foodborne outbreaks associated with norovirus, when clinical or food samples are not available; and assessment of the effectiveness of environmental or hand hygiene practices in food handling as described in Codex CAC/GL 79-2012. The proposed symposium will focus on the latest developments in surface sampling methods specifically for recovery of human norovirus, and how these methods are being used to assess and control contamination.

S18 Viruses and Parasites on Produce: Challenges and Opportunities Farm to Fork

ROBERT TAUXE: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
HARI DWIVEDI: *bioMérieux, Inc., Hazelwood, MO, USA*
ALEXANDRE DASILVA: *U.S. Food and Drug Administration, Laurel, MD, USA*
ANGELA FRASER: *Clemson University, Clemson, SC, USA*

As consumer demands for ready-to-eat fresh produce rise, so does the risks for foodborne illnesses. Emerging viruses and parasites are responsible for an increasing number of outbreaks each year. Norovirus, the most common cause of foodborne illness outbreaks in the United States, is responsible for approximately 20 million illnesses a year, resulting in hundreds of deaths, and *Cyclospora cayetanensis*, the culprit behind last summer's cilantro outbreak, infected more than 500 persons. As a vehicle for pathogens, raw produce poses a greater threat to public health safety than many other commodities, as it lacks a "kill" step such as cooking that prevents transmission. Due to an absence of validated detection methodologies and reported epidemiology of viruses and parasites, the focus in the past has been on contamination caused by bacteria. By nature, viruses and parasites are more difficult to detect, and detection and surveillance strategies are immature compared to those of bacteria, creating challenges for industry and public health professionals. As viral and parasitic detection science improves, action must be taken to protect consumer health by preventing transmission, highlighting the need for sound education and extension training at all levels of the produce food chain. Viruses and parasites share many characteristics in terms of epidemiology, detection, and control, suggesting joint lessons can be learned from exploring the challenges posed by each. This symposium will discuss the epidemiology of viral and parasitic produce contamination, and will address the current challenges, advances, and opportunities in the field of detection and prevention of produce-transmitted viral and parasitic pathogens.

S19 Novel or Rapid Sampling Methods for Utilization in Slaughter and Processing Establishments

MELANIE ABLEY: *U.S. Department of Agriculture-FSIS, Washington, D.C., USA*
TOMMY WHEELER: *U.S. Department of Agriculture-ARS-USMARC, Clay Center, NE, USA*
ANGELA SIEMENS: *Cargill, Wichita, KS, USA*
MOHAMMAD KOOHMARAIE: *IEH Laboratories and Consulting Group, Seattle, WA, USA*

Recent illness outbreaks have shown outbreak strains can persist in slaughter and raw processing, in addition to RTE establishments. There are increasing developments in the novel and rapid sampling methods FSIS regulated establishments can use to verify the effectiveness of their food safety systems. Under Hazard Analysis and Critical Control Point (HACCP) regulations, FSIS regulated establishments are required to control food safety hazards and conduct verification activities (which may including sampling) to demonstrate that their systems are working as intended. Additionally, FSIS regulated establishments are required to develop Sanitation Standard Operating Procedures (SSOP) to describe daily procedures to prevent direct contamination or adulteration of products. Cost effective, rapid, and less labor intensive methods can aid establishment's verification activities to ensure the HACCP system is functioning as intended on an ongoing basis. This symposium will bring together speakers from academia, industry, and government to present new trends in sampling methods. FSIS regulated establishments may be able to utilize those sampling methods to support their verification activities under their HACCP system and sanitation programs to comply with FSIS regulations and reduce the risk of foodborne illness from their products.

S20 Viable But Non-culturable (VBNC) Bacteria: Not Your Typical Injured Cells

JAMES OLIVER: *University of North Carolina at Charlotte, Charlotte, NC, USA*
BILL KEEVIL: *University of Southampton, Southampton, United Kingdom*
LAURA GAGE: *Albemarle Corporation, Baton Rouge, LA, USA*

Despite the many expectations, PCR-based technologies in food diagnostics have not replaced standard culture methods with isolates still being necessary for epidemiology and law enforcement. Many molecular methods available today require a cell density of at least 10^3 to 10^4 CFU/ml of the target pathogen. Consequently, the successful recovery and detection of foodborne pathogens continue to depend on the bacterial ability to grow and multiply while competing with the background microflora. In the food processing chain, foodborne pathogens are subjected to various unfavorable environmental conditions that can lead to the formation of injured stressed cells and viable but non-culturable (VBNC) cells. Both physiological states in the bacterial response to physical and chemical stresses should not be confused; sublethally injured cells can repair themselves and return to a normal physiological state under favorable conditions; VBNC typically do not form colonies on rich media, despite being viable, yet they can resuscitate in vivo and cause disease. For risk assessment studies, it is, therefore, critical to recover, when possible, all forms of stressed cells during culturing procedures. With the support of the Applied Laboratory Methods PDG, the Meat and Poultry Safety and Quality PDG, the Food Hygiene and Sanitation PDG, the Pre-Harvest Food Safety PDG and the Water Safety and Quality PDG, the symposium will provide, in three presentations, the latest information on the induction as well as in-vivo and in-vitro resuscitation of VBNC organisms including *Escherichia coli* O157:H7, *Salmonella*, *Listeria*, *Vibrio* and *Legionella*.

S21 Cyanotoxins in the Water Supply and Potential Food Safety Ripple Effects

KELLY MAGURANY: *ConAgra Foods, Naperville, IL, USA*
LESLEY D'ANGLADA: *U.S. EPA, Washington, D.C., USA*
RICHARD LORENZ: *Ohio State University, Westerville, OH, USA*

Microcystin is a type of cyanotoxin and is one of the several toxins produced by the cyanobacteria otherwise known as blue-green algae. The presence of high concentrations of microcystin in potable water recently became a serious health risk in the United States with potential impact on the food industry. Increasing levels of cyanotoxins in water has also been recognized as an emerging global issue with potential implications for irrigation water used in agriculture. The combination of increased surface water temperatures and increased nutrient levels from farm and waste water run-off are expected to be the primary drivers for increased algae blooms. Both the U.S. EPA and the World Health Organization have devised advisory or provisional guidance values for microcystin in potable water supplies to support water monitoring. As algae blooms continue to grow in severity presenting an environmental challenge not just in the United States but globally, there is a need for increased research and awareness on cyanotoxins as well as effective mitigation programs and development of analytical methods to adequately identify each toxin. This information will be beneficial in supporting global public water system managers who are on the front lines of this issue, ensuring that our potable water supply continues to be safe for human

consumption and use in food manufacture. In this symposium, we will review cyanotoxins and their effects on human health, potential impacts to the food industry including potable water used in food production and irrigation for agriculture, discuss monitoring activities, occurrence rates, action levels set by global regulatory agencies, and explore applicable mitigation methods.

S22 Analysis of Gluten in Foods: Where are We and Where Do We Need to Go?

CARMEN DIAZ-AMIGO: *Food Allergen Consultant, Hamburg, Germany*
RAKHI PANDA: *U.S. Food and Drug Administration, College Park, MD, USA*
TERRY KOERNER: *Health Canada, Ottawa, ON, Canada*
MELANIE DOWNS: *University of Nebraska-Lincoln, Lincoln, NE, USA*
ROLAND ERNEST POMS: *MoniQA Association, Neutal, Austria*

Approximately 1 in 141 people in the US are affected by celiac disease and adherence to a strict gluten-free diet is the only option to prevent inflammatory symptoms in sensitive individuals. In 2013, the FDA issued a regulation requiring food bearing the claim "gluten-free" must contain less than 20 ppm (mg/kg) gluten. Accurate detection and quantification of gluten are essential to support regulatory requirements, whose ultimate goal is the prevention of adverse reactions in gluten-sensitive individuals. Enzyme-linked immunosorbent assay (ELISA) remains the method of choice by the food industry and the regulatory agencies for the analysis of gluten in foods. Although several commercial ELISAs are available and useful in accurately detecting and quantifying intact gluten present in foods, the accuracy of the available ELISAs in quantifying hydrolyzed and fermented gluten is questionable. Mass spectrometry (MS) is emerging as an alternative analytical method for gluten analysis and has the potential to solve some of the problems encountered by ELISAs. This symposium will present an overview of the usefulness and the limitations of the currently used ELISA methods for accurate detection and quantification of gluten in foods. The use of advanced MS-based methods for gluten analysis will also be discussed. The session will close by highlighting and discussing effective analytical strategies that are necessary for the validation of gluten-free programs essential for the protection of sensitive individuals.

S23 Prokaryotic Hibernators – Persisters in Foods – What is Really Going on?

SEAMUS FANNING: *University College Dublin, Dublin, Ireland*
KIERAN JORDAN: *Teagasc, Fermoy, Cork, Ireland*
AUTUMN BROWN GANDT: *Northeastern University, Boston, MA, USA*

Old nomenclature would refer to bacterial pathogens in certain food environments and conditions as cells that are undergoing stress or may be injured cells. Current reference to these types of cells would include persisters or prokaryotic hibernators. Although much is not understood on their mechanism of survival in foods for prolonged periods of time, current research has started to shed some light on how these bacteria respond to these harsh environments and perhaps how they shut down their physiological systems. Of concern to food safety and public health is that these "physiologically dormant" pathogens may not be recovered during routine food analyses yet are capable of remaining pathogenic and still lead to disease. The elucidation of the genetic factors involved with the bacteria that allows them to remain a viable pathogen yet still lead to disease is critical and may also lead to the identification of important biochemical conditions, e.g. enrichment broths, which can restore growth capabilities of the organism. This may facilitate the isolation of once difficult or unculturable bacteria so that a more accurate analysis can be determined. The actual contribution to foodborne outbreaks and illness of prokaryotic hibernators/persisters is unknown but may have a significant impact on food production and food safety. This symposium brings together speakers who will discuss how foodborne pathogens, including *Salmonella*, *Listeria*, and *Cronobacter*, adapt to unsupportive environments, such as powdered infant formula, and provide current research at the molecular/genetic level on the change in physiological state of the prokaryotic hibernators/persisters.

S24 Pathogen Adaptation: Transmission from the Environment to Host and from Host to Host

SLAVA EPSTEIN: *Northeastern University, Boston, MA, USA*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*
MAIRE BEGLEY: *Cork Institute of Technology, Cork, Ireland*
ANDREAS BAUMLER: *University of California-Davis, Davis, CA, USA*

Globally, as the human population continues to increase, the interaction and contact between people, animals, and our environment become greatly amplified, introducing the risk of exposure to new foodborne viruses, bacteria, and other disease-causing pathogens and becomes more significant and impactful. The convergence of these three sectors has created a new paradigm in which the health of each group is inseparably interconnected and totally interdependent. Advancing technologies such as whole genome sequencing and science-based evidence is increasing the awareness, knowledge, and understanding of this interdependence of the health of humans, animals, and the environment. However, attentive safeguarding of our food and feed supplies from food-borne pathogens is vital for human, animal, and environmental health. To improved public health and food safety measures, the development, implementation, and sustainment of a national strategy based on the knowledge obtained from such studies is needed to understand this profound interdependence and realization that we are part of a larger, exquisite, and elaborate ecological system. The critical aspect of a pathogen's ability to colonize a host and proceed to generate virulence factors to lead to infection. Worldwide, food is a common vehicle for pathogens transmission, either from the environment directly via plant products or food animals. This symposium will explore the molecular dynamics that affords the pathogen the ability to adapt, survive, persist, and grow in its initial environment and yet retain genetic information to remain a human pathogen. In some cases, specific genes are either mutated or deleted from an ancestral strain providing a better fit for the pathogen. Gaining a better understanding of these genetic mechanisms and their evolutionary driving force may expand the tools that the food industry and regulatory agencies need to reduce the opportunities of pathogens to find their way into the food supply.

S25 Multiplex Foodborne Pathogen Detection Assays: Fishing for Them All with One Bait

HARI DWIVEDI: *bioMérieux, Inc., Hazelwood, MO, USA*
MICK BOSILEVAC: *U.S. Department of Agriculture-ARS-U.S. Meat Animal Research Center, Clay Center, NE, USA*

Rapid detection methods for foodborne pathogens have been significantly used by the food industry for their routine testing programs for more than 25 years. Meantime, the food industry has required higher specificity and sensitivity for these methods. Detection technologies have moved from single target to multi-target assays in order to enhance assay specificity towards the most virulent bacterial genera/species and to reduce the risk of detection of non-target bacteria. Both DNA/RNA- and antibody-based assays are now offering multiplex detection systems, and can be used not only for detection but also identification/confirmation of bacterial isolates, contributing to facilitate and to shorten these latter steps. They may also be applied for the simultaneous detection of different pathogenic bacteria in foodstuff.

This session will present the state of the art of multiplex assay based methods in regards to their routine use for the detection and identification of foodborne pathogens. Field examples will illustrate the objectives of such assays, their workflow, their strengths and weaknesses for food testing in order lab operators and managers better the performances of those assays.

S26 Advances in Portable Devices for Food Protection and Defense

KENNY XIE: *United States Pharmacopeia, Rockville, MD, USA*
SANJEEWA KARUNATHILAKA: *U.S. Food and Drug Administration, College Park, MD, USA*
PETER PALMER: *San Francisco State University, San Francisco, CA, USA*
ISMAIL BOYACI: *Hacettepe University, Ankara, Turkey*

With an increasingly globalized market, rapid and sensitive detection of authentication and adulteration of foods and food products is essential for protecting consumers. Recently, priority has been placed on developing screening methods that employ portable, rapid, high-throughput instrumentation with a goal of preventing illnesses and adverse effects from either intentional or unintentional contamination. These portable devices could allow for sample analysis and triage outside traditional laboratory settings by both regulators and manufacturers in order to improve the food safety of consumer products. While portable devices are more established in the pharmaceutical arena, their use in the evaluation of foods and dietary supplements is increasing. This symposium brings together representatives from government, industry, and academia to showcase the most recent advances in high priority food commodities with both established and emerging portable technologies. Specific presentations focus on milk powders (e.g., melamine/nitrogen contamination), food supplements (e.g., heavy metals and safety concerns), meat (e.g., species substitution and risk of disease), and olive oil (e.g., substandard oils and potential allergenicity) and using near infrared spectroscopy, Raman spectroscopy, X-ray fluorescence detection, and laser-induced breakdown spectroscopy.

S27 Strengthening the Hazard Analysis of Food Safety Plans

BENJAMIN WARREN: *Land O' Lakes, Inc., Arden Hills, MN, USA*
JOSEPH MEYER: *The Kraft Heinz Company, Glenview, IL, USA*
TIMOTHY ADAMS: *The Kellogg Company, Battle Creek, MI, USA*

A comprehensive and well-defined hazard analyses can serve as the backbone in assuring the safety of the food supply in the global market. There will be no hazard to manage and/or control if a hazard is not properly identified or is not known to occur. Common deficiencies in hazard analysis include, but are not limited to, non-identification of significant hazards due in part to an inaccurate flow diagram, lack of scientific basis for the identification of the food safety hazard, inadequate justification for decisions made on their significance during food production, and insufficient data to support the design of preventive controls. In this mini-symposium, speakers will lead the discussion on how to reassess the hazard analysis, and offer insights on how to effectively enhance both the ingredient and process hazard analyses and where/how to obtain scientific justification for the identified hazards. The importance of utilizing data to drive sound, science-based decisions on hazard analysis will be emphasized. This interactive session will also be designed to generate audience participation using technology, such as smartphones or other handheld devices.

S28 Update on Implementation of the Food Safety Preventive Controls Alliance Training

ROBERT BRACKETT: *Illinois Institute of Technology, Bedford Park, IL, USA*
KATHERINE MJ SWANSON: *KMJ Swanson Food Safety, Inc., Mendota Heights, MN, USA*
JENNY SCOTT: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*

The Food Safety Preventive Alliance (FSPCA) was established in 2011 to help small and midsize food companies to comply with the Preventive Controls Rules. With the finalization of these rules for human and animal foods, the FSPCA has officially implemented training. This session will detail what is included in the training, how individuals can access the training, and how individuals can become Lead Instructors. In addition, the session will provide information on how food safety experts can become part of and benefit from, the National Technical Training Network that supports FSPCA and FDA training.

S29 A Case Study Covering *Salmonella* Newport in the Delmarva Peninsula

ERNEST JULIAN: *Rhode Island Department of Health, Providence, RI, USA*
ERIC BROWN: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
LAURA STRAWN: *Virginia Tech, Painter, VA, USA*

The Centers for Disease Control and Prevention estimates that approximately 1.2 million illnesses occur due to non-typhoidal *Salmonella* annually in the United States. In the majority of produce-related outbreaks, it is difficult to determine the definitive cause and route of transmission. Since 2002 outbreaks of *Salmonella* Newport have been associated with tomatoes and cucumbers grown and harvested in the Delmarva region of the East Coast of the United States. Epidemiological evidence supports these finding and makes this area a critical case study for learning and following disease transmission. Research conducted by the FDA and extension specialists in Delmarva provides evidence that this specific strain of *S.* Newport strain 061 has been found in wildlife, tomatoes, irrigation water, surface water and sediment. This session will cover the historical findings of the unknown reservoirs of *S.* Newport strain 061 in the Delmarva area, using epidemiologic and environmental findings, grower best practices, and the basic scientific findings in an attempt to cure an endemic contamination issue. The curious case of the Delmarva describes a unique environment with what is perhaps not a unique problem, that is an undetermined reservoir for *Salmonella* and an engaged community of growers, public health advocates, and researchers. This session will describe many of the efforts taken over the past 10 or so years by growers and researchers and the past year by the active Delmarva Task Force.

S30 Surrogate for Low-moisture Foods Validation: What are the Key Steps from Selection to Routine Use?

GARY ACUFF: *Texas A&M University, College Station, TX, USA*
JEFF KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*
PABLO ALVAREZ: *Novolyze, Dijon, France*

Nowadays, multinational food companies, as well as medium and small food producers, face up a real issue regarding the variety and complexity of processes to be validated before final adoption. Furthermore, they also face the diversity of the type and origin of food matrixes and the various raw materials to be used. Therefore, it is necessary to pay particular attention to the verification programs for foreign suppliers and the compliance with US food safety rules. The compliance requirements with FSMA rules have put in evidence the limits of the current process validation strategies and thereby the need to develop new specific and well-characterized surrogates. Surrogates dedicated to the validation of food process have been in use for many

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years, such as *Enterococcus faecium* NRRL B-2354 for the validation of the almond roasting steps. Nevertheless, if surrogates need to be applied to other process validation and other food types, it is important to better define the requirements. A good surrogate can be only defined after having completed a qualification program taking into account a number of variables: the process to be validated, the food matrix, the thermal resistance of the surrogate in comparison with a specific pathogen, stability and its capacity to be implanted in the matrix.

S31 The Rise of the Genomes – Improving Health through Better Food Quality and Food Safety

PETER GERNER-SMIDT: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
DEANN AKINS-LEWENTHAL: *ConAgra Foods, Omaha, NE, USA*
ROBERT C. BAKER: *MARS Incorporated, McLean, VA, USA*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*
VINCENT B. YOUNG: *University of Michigan, Ann Arbor, MI, USA*
GARY WU: *University of Pennsylvania, Philadelphia, PA, USA*

This session follows up on the past ILSI North America sponsored, "Rise of the Genomes," sessions in 2014 and 2015; with a focus on the applications of next generation sequencing methods, including whole genome sequencing for microbial source tracking, RNA-seq and metagenomics. Whole genome sequencing is becoming a routine tool to track foodborne pathogens from the farm to the table to identify and control outbreaks quickly and efficiently. The tool is also increasingly used by the industry to track pathogens in the production environment and characterize beneficial bacteria used in the food production, e.g., starter cultures and probiotics. However, bacteria in the gut are not just associated with foodborne infections. The composition of the gut flora, the gut microbiome, is also related to illness, e.g., cancer and atherosclerosis and the composition of the microbiome is dependent on and may be altered through dietary changes. Next generation sequencing is also used to study the microbiome through metagenomics and RNA-seq can be applied to study how a microorganism thrives in a given niche.

This symposium will include an overview of the application of next generation sequencing, Two talks by representatives of the food industry on their experiences using whole genome sequencing to improve food and food safety, a talk on the use of RNA-seq to elucidate genetic elements and pathways used by pathogens to thrive in food environments, and talks about the use of metagenomics to study the impact of the microbiome on health.

S32 Tailoring Acceptance Sampling Theory for Enhanced Microbial Risk Management

ROBERT BUCHANAN: *University of Maryland, College Park, MD, USA*
MARCEL ZWIETERING: *Wageningen University, Wageningen, Netherlands*
MOEZ SANAA: *ANSES, Maisons-Alfort, France*
URSULA GONZALES-BARRON: *CIMO Mountain Research Centre, School of Agriculture (ESA), Polytechnic Institute of Braganza (IPB), Braganza, Portugal*
VASCO CADAVEZ: *CIMO Mountain Research Centre, School of Agriculture (ESA), Polytechnic Institute of Braganza (IPB), Braganza, Portugal*
ANTONIO VALERO: *University of Cordoba, Cordoba, Spain*

Microbial sampling is a risk management strategy used to evaluate whether a food safety system is correctly implemented. Although microbiological sampling cannot guarantee with 100% certainty the safety of food products, still it is commonly accomplished to comply with regulatory microbiological criteria or to assess whether food production processes are under control. Sampling plans have been conventionally derived by borrowing concepts of acceptance sampling theory from classical quality statistics. However, the classical simplistic assumptions of data normality and homogenous contamination among production batches have been demonstrated to affect the efficacy of the sampling plans. Since within-batch testing regimes are critical in the sense that they aid in determining whether food safety targets are being achieved, sampling plans should be designed using sound statistical methods that ensure the desired level of protection. Thus, in the past few years, two phenomena known to strongly impact on the performance of a sampling plan have been studied: the spatial clustering of bacteria cells in foods and the heterogeneity in microbial contamination among batches. To address these issues, there have been some efforts in the investigation of other statistical distributions to better represent bacterial clustering, and a situation originated thereof, the high proportion of zero counts in samples, especially when contamination levels are low. Nevertheless, whatever the statistical distribution of microorganisms is chosen, considering the between-batch variability in microbial contamination is relevant for making some safety allowance for the effectiveness of sampling plans. In addition, some researchers have also proposed the use of past monitoring microbial data in order to establish realistic tolerance criteria, as well as the development of new methodologies, if possible Bayesian, for updating the sampling plans, as new sampling data are collected. Thus, the objective of this symposium is to discuss the weaknesses of traditional acceptance sampling. For the design of new-generation sampling plans, speakers will review new concepts and trends that are more informative, dynamic and amenable to be updated, and hence, more efficient in ensuring the achievement of food safety risk targets.

S33 Food Safety 2050: A Glimpse into the Future

ISABEL WALLS: *U.S. Department of Agriculture-NIFA, Washington, D.C., USA*
J. GLENN MORRIS: *University of Florida, Gainesville, FL, USA*
JIE XU: *Georgia Technology Research Institute, Atlanta, GA, USA*
SEAN LEIGHTON: *The Coca-Cola Company, Atlanta, GA, United States*

What does the future of food safety look like? This session, encompassing industry, academic, and governmental professionals, takes a multi-generational approach to explore the future of food safety. Experienced and upcoming professionals will envisage future challenges and what must be done to prepare ourselves. The session will explore futuristic technologies, such as inventive pathogen/chemical detection and preventative methods. Experts will provide insight on climate change, its effects on the emerging and novel pathogens, pathogenicity and how should the industry prepare itself to face these challenges. Which emerging hazards will impact us the most: super-bugs, chemical contaminants, radiological events, food fraud, or new and unforeseen allergens? Garnering advice from individuals who have spent the better part of their careers in food safety, to those who are just embarking on what will surely be a fascinating career in food safety—Food Safety 2050 will give us a glimpse of what's in store. This session will directly address many aspects of what the future will hold: Climate change and food safety; Predicted emergence of new or unexpected hazards; Exploration of futuristic technologies; Regulatory considerations; Advice from seasoned food safety professionals for the next generation; How the future will reshape our infrastructure and ability to access resources; and Long-term strategic planning. This session will hear from experts who have already begun to think about the future and what it will look like in terms of food safety. They will present mitigation strategies for protecting the global food supply and highlight exciting breakthroughs that will give us an advantage. Attendees will garner knowledge, share best practices for innovative solutions and will discover what the future may hold for those in our industry.

S34 Crowdsourcing and Novel Digital Data: 21st Century Tools for Food Safety Monitoring, Surveillance, and Management

PATRICK QUADE: *iwaspoisoned.com, New York, NY, USA*
ELAINE NSOESIE: *University of Washington, Seattle, WA, USA*
JAMES KAUFMAN: *IBM Almaden Research Center, San Jose, CA, USA*

The digital revolution has changed the availability of data related to food consumption, consumer patronage of food establishments and food safety incidences linked to consumer activities. One such source of online monitoring is through crowdsourcing by utilizing popular social networking sites (i.e.: Twitter, Facebook, GrubHub, Foursquare, and Yelp) to gather consumer experiences at retail establishments in real-time. Additionally, the development of dedicated resources such as smartphone apps and websites (like the disruptive site IWasPoisoned.com) that allow consumers to directly share adverse food experiences provide other means of valuable crowdsourced data. Such reports and reviews have the potential to be utilized as novel additional sources of information for routine foodborne outbreak surveillance. Not surprisingly, careful filtering through a large amount of social "noise" and consideration of actual disease etiology (i.e. incubation period, symptoms, etc.) are necessary to render such information useful. Furthermore, a proper degree of social participation in sharing and reporting adverse experiences regarding food also needs to occur. Another digital source of real time food consumption information that can be utilized to aid outbreak investigation is food sales data. However, utilizing all of these tools presents multiple potential pitfalls, as many considerations are required for them to be effective.

In the proposed symposium, considerations regarding these newly available tools to supplement and monitor food safety event data will be discussed by global experts in food safety and extension followed by a brief open discussion with audience interaction.

Discussion will include:

I) filtering and discriminating relevant crowdsourcing information for effective use

II) the degree of participation required for these tools

III) maintaining both corporate and individual privacy

IV) the specific type and form of real time food safety data through traditional surveillance routes required for these tools

V) challenges in digital food safety management

S35 From Cow to Cup: How Dairy Industry Stakeholders Manage Risks of Drug Residues

PATRICK GORDEN: *Iowa State University, Ames, IA, USA*
ROGER HOOI: *Dean Foods, Dallas, TX, USA*
ROBERT SALTER: *Charm Sciences, Inc., Lawrence, MA, USA*

Among the measures available to treat and prevent disease in dairy cattle, the judicious and responsible use of antibiotics and other drugs by veterinarians and producers can have a positive impact on animal health while maintaining a safe milk supply. Whereas the use of such drugs is highly regulated, the potential for drug residues in milk and meat exists if they are incorrectly used. Veterinarian-client-patient relationships, education and preventive programs reduce the risk of residues. Beyond the farm, all milk received at dairy processing facilities in the U.S. is tested for the presence of antibiotics, as prescribed under Appendix N of the Grade "A" Pasteurized Milk Ordinance to prevent adulterated milk from entering commerce. Beta-lactam antibiotics, the most commonly used drugs on dairy farms, are the primary target of this testing. Under the Grade "A" program, the National Milk Drug Residue Database has reported decreasing incidence of actionable residues in milk; only 0.014% of tankers tested were positive in 2014; no positives were reported in pasteurized dairy products. Recently, questions have risen about expanding mandatory testing programs to other drug residues. Under the direction of the National Conference on Interstate Milk Shipments, FDA developed a modeling tool to assist in risk management of drug residues in milk products. The outcome, published early 2015, provided a risk-ranking of selected drugs aiding the development of a framework for additional testing and monitoring of Grade "A" milk. This symposium will provide an overview of current drug use on the farm and programs designed to reduce the risk of drug residues in the milk supply. The outcome of the drug residue risk-ranking and its implications on industry stakeholders will be discussed in regard to current and future testing and control programs, and the development of new methodologies for identifying additional drug residues in milk.

S36 Competent People Doing Comparable Work: Developing Food Protection Professionals on a Global Scale

CRAIG KAML: *International Food Protection Training Institute, Battle Creek, MI, USA*
LESLIE BOURQUIN: *Michigan State University, East Lansing, MI, USA*
CHARLES MUYANJA: *Africa Association for Food Protection, Kampala, Uganda*
HERNA GERBA: *Ethiopian Food, Medicine, and Healthcare Administration and Control Authority, Addis Ababa, Ethiopia*

Globalization of food trade demands a competent and comparably trained food protection workforce throughout the world. Disparities in food protection knowledge and skills across various levels in the food industry and government, as well as, between countries and regions of the world, create significant barriers to ensuring a safe global food supply.

Developing comparable skills and talent across the broader food safety workforce is a challenge. The challenges can include limited resources, lack of competencies from which to build curriculum, lack of standardized curriculum, and alignment with the needs of the target audience. These challenges are universal across the food system for both industries (food producers, manufacturers, and food service) and government (regulators and inspectors). These challenges can be even more pronounced in low and middle-income countries, where the food safety workforce is unable to get the training they want and need in order to increase their capacity. Food safety programs from such countries often must rely on donor-driven training programs (such as FAO, UN, WTO) to address minimal needs. However, there are a growing number of examples of innovative and successful initiatives in training, education, and workforce capacity building across food production, government, industry, and academic sectors. Collaboration between these sectors is key in optimizing the safety of the food supply of any country as each has a role to play, and can learn from the others.

This session will present successful programs, explore available resources, examine challenges, and call for the need for a trans-national competency-based learning framework to build a strong global food protection workforce.

S37 Mitigating Intentional Adulteration: What You Should be Doing Today

COLIN BARTHEL: *U.S. Food and Drug Administration, College Park, MD, USA*
AMY KIRCHER: *Food Protection and Defense Institute, St. Paul, MN, USA*
LANCE REEVE: *Nationwide Insurance, Columbus, OH, USA*
JOHN LARKIN: *Food Protection and Defense Institute, St. Paul, MN, USA*

Throughout history, food has been used as a delivery mechanism to cause catastrophic public health harm. Today this threat remains, as intentional adulteration plots are uncovered with surprising frequency. Due to the nature of the globally distributed and highly integrated food systems, rapid identification of an assault to the system is an ongoing challenge. Intentional adulteration events and scenarios are of grave concern to the U.S. and global populations as food is one infrastructure that nobody can opt out of.

With the release of the FDA rule on Intentional Adulteration* there are critical elements you should be planning now. This session targets the key activities a food company should be doing now to mitigate against intentional adulteration. This session highlights the critical work that must occur to prepare food companies to detect and mitigate an intentional adulteration event in the food system. Specifically, presenters will highlight the recently released FDA rule on Intentional Adulteration, understanding your supply chain, demonstrate importance of and how to conduct vulnerability assessments, consider reasonably foreseen potential threats, develop you own food defense plan, harden your firm against such attacks and what education/training is necessary to become a qualified individual.

*The FDA rule on Intentional Adulteration is scheduled for release on May 31, 2016

S38 Food Defense Lessons Learned from the 2015 U.S. Avian Influenza Outbreak

DAVID SCHMITT: *Iowa Department of Agriculture and Land Stewardship, Des Moines, IA, USA*
STEVE OLSON: *Minnesota Turkey Growers Association, Buffalo, MN, USA*
LINDSEY GARBER: *U.S. Department of Agriculture:APHIS:VS:STAS:CEAH:M&M, Fort Collins, CO, USA*

In 2015, the U.S. experienced an outbreak of highly pathogenic avian influenza (HPAI) in commercial poultry flocks throughout the country, with profound impacts in the Midwest, most notably Iowa and Minnesota. This symposium will explore the effects of the 2015 HPAI outbreak on the U.S. poultry industry, and how both industry and government have adapted best practices and response plans based on experience to better defend the poultry industry from future infectious disease outbreaks, both natural and intentional. The three speakers will address the outbreak from the perspectives of industry, state government and federal government covering both the outbreak in Minnesota and Iowa. They will discuss immediate and long-term impacts to the poultry industry of the outbreak, with a particular emphasis on biosecurity and other production-based changes. Additionally, they will discuss those challenges faced by government responders, including personnel capacity (surge) for control measures, and depopulation and response timelines.

S39 A Map to a Safer Future: Applications of Geographic Information Systems and Remote Sensing for Food Safety

DANIEL WELLER: *Cornell University, Ithaca, NY, USA*
JENNIFER QUINLAN: *Drexel University, Philadelphia, PA, USA*
MARK BRUHN: *RTI International, Research Triangle Park, NC, USA*

While opportunities for the application of GIS-RS exist throughout the farm-to-fork chain, relatively few food safety specialists use GIS-RS. In fact, much of the GIS-RS-based food safety research is being conducted by individuals outside the IAFP community (e.g., ecologists). Despite its widespread use in other fields, GIS-RS is therefore underutilized by food safety specialists. This symposium will discuss (1) different ways GIS-RS is currently being applied throughout the farm-to-fork chain and in outbreak response, (2) potential applications of GIS-RS for food safety, and (3) the utility of GIS-RS for big data management. The proposed symposia are, therefore, an ideal follow-up to the Big Data Symposia held at IAFP 2014.

GIS-RS is a powerful tool for data collection, management and analysis. For example, researchers in academia are using RS data in GIS platforms to predict the risk of microbial contamination in production environments. Similarly, government agencies use GIS to improve food traceability and investigate outbreaks. However, these applications do not fully utilize the data management, and analysis capabilities of GIS-RS, especially when compared to other scientific fields. For example, precision agriculture uses GIS to maximize crop yields while minimizing capital inputs. However, the same data that is being generated for these analyses could also be used to develop targeted, adaptive pre-harvest risk management plans. GIS-RS also has tremendous potential to model and predict the effects of climate change on food safety. The use of GIS-RS will require a shift toward interdisciplinary collaborations between academia, government and industry, and will require the standardized collection and sharing of data. Food safety specialists from all three facets of food safety, therefore, need to have an understanding of GIS-RS. This symposium will work to bridge the gap between GIS-RS practitioners and food safety specialists, while exploring current and potential applications of GIS-RS to food safety.

S40 "If I can't pronounce it, I'm not eating it!" How Consumer Perceptions are Changing the Face of the Food Industry

JANEAL YANCEY: *University of Arkansas, Fayetteville, AR, USA*
SANDRA FURBEE: *Nestle, Solon, OH, USA*
JUSTIN RANSOM: *McDonald's, Aurora, IL, USA*

Products intended for retail and food service are formulated with input from a company's product development, regulatory, procurement and quality assurance teams. Once these developed products reach consumers, the list of ingredients often have names that are difficult to pronounce and unknown. When this occurs, some consumers pour over the fine-print; heeding only that the list is sometimes populated by non-essential, "scary" ingredients. Some wrongfully conclude that these complicated-sounding, sometimes un-pronounceable, named components must be artificial or unnatural and are therefore toxic to the body. This misconstrued information is compounded by (often uninformed) food bloggers and television doctors, as well as the news media who look for a shock-value.

Today's consumers have expanded their definition of quality to also include products that make them feel good about what they are buying. As such, Mintel research has shown increasing trends towards minimally processed, natural, non-GMO, preservative-free, and/or organic products. Consumers are also demanding a higher degree of transparency from the food supply chain to truly understand what is in their food and where it comes from. With advancements in social media and global communications, consumers are exerting increasing pressure on the food industry to provide them with a new definition of "clean label."

As a result, food scientists are working hard to meet the demands of consumers while still providing a cost-effective, minimally processed, safe product that still tastes great. These efforts have also migrated to the marketing departments to ensure that these new initiatives are transparent.

This session will directly address:

- Understanding more about current perception trends and what is driving these trends.
- Take a deep dive into the clean label initiative.
- Take a look at one company's transparency campaign. Understand public response and what they've changed as a result.

S41 Now That Whole Genome Sequencing Has Arrived, What Does the Data Really Tell Us?

PALMER ORLANDI: *U.S. Food and Drug Administration-CFSAN, Silver Spring, MD, USA*
DAVID LACHER: *U.S. Food and Drug Administration, Laurel, MD, USA*
JOHN BESSER: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
TIM FREIER: *Merieux NutrisSciences, Minnetonka, MN, USA*

The utility and application of Whole Genome Sequencing have made significant progress for its acceptance and use in different aspects of food safety programs. As the technology advances and becomes a more practical, less expensive means to ensure the global food supply is less likely to harbor pathogenic microbes, there remain several issues that the food safety community should address. To avoid the debate as to whether food contains a live or dead (or infectious or non-infectious) non-culturable microbe, one topic that has surfaced over the past years that requires further discussion is what data determines the link between different isolates, i.e., how many SNPs determine a new/different species of the same strain. As with PFGE there were certain 'rules' that were followed that allowed the determination of isolates were clones or not, WGS may need a similar set of ground rules. The objective of this symposium/roundtable is to bring the issue forward and initiate the appropriate discussions to move forward, perhaps with the establishment of working group to explore possible guidelines that in the near future be brought to the membership of the ALM and AMA PDGs. This critical point of WGS analysis has reverberation throughout the entire spectrum of users of this technology and its effect on food safety.

S42 Next Generation Sequencing, Food Safety, and What It Means to the Food Industry and Food Regulators

MARC ALLARD: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
JOHN BESSER: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
BALAMURUGAN JAGADEESAN: *Nestec SA, Nestle Research Center, Lausanne, Switzerland*
LEEN BAERT: *Nestlé Research Center, Vers-chez-les-Blanc, Switzerland*

Over the past few years, advanced molecular technology is creating a sea of change in the operations of public health surveillance for foodborne illness and the tracking of foodborne contamination. This symposium will be a summit of industry food safety professionals and representatives from at least two of the Federal agencies now using next generation sequencing for surveillance of foodborne illnesses and investigation of food contamination events from a regulatory perspective. The focus of the presentations will be providing an overview of how next generation sequencing is changing the PulseNet model for public health surveillance and what those changes will mean to the food industry. Attendees will learn:

- How the U.S. CDC PulseNet model is changing to include whole genome sequencing (WGS) as a replacement for pulsed-field gel electrophoresis (PFGE) for the surveillance of foodborne pathogens
- Why the U.S. FDA GenomeTRAKR program has implemented WGS for the tracking of foodborne contamination in foods and food environments and how those changes have impacted regulatory operations
- How some parts of the food industry are using WGS to track foodborne contaminants through their supply chain
- What concerns industry has about the use of the new technology and regulatory implications

S43 How Do I Validate That? Assuring Credibility of Non-thermal and Novel Thermal Controls for Microbiological Hazards

NATHAN ANDERSON: *U.S. Food and Drug Administration-IFSH, Bedford Park, IL, USA*
KATHLEEN GLASS: *University of Wisconsin-Madison, Madison, WI, USA*
JEYAMKONDAN SUBBIAH: *University of Nebraska-Lincoln, Lincoln, NE, USA*
BRENDAN NIEMIRA: *U.S. Department of Agriculture-ARS, Wyndmoor, PA, USA*

Validation of hazard control measures is an important requirement of food safety management systems, and FSMA is amplifying the urgency of such validations. However, the vast majority of prior work and information available has focused primarily on reduction and control of microbial pathogens via thermal processes. In contrast, criteria for the design, execution, and interpretation of validation studies for non-traditional/non-thermal controls often are unclear or simply unavailable. A wide-range of products are subjected to process steps that provide pathogen reduction by solely non-thermal means, such as high-pressure processing, varied wavelength light exposure, and/or radio frequency treatment. This session discusses approaches to validation of a selection of non-thermal control measures.

S44 Updating Our Knowledge in Cold Chain Management: Challenges and Solutions in International Supply Chains

ROLF IBALD: *European University of Applied Science, Bruhl, Germany*
PANAGIOTIS SKANDAMIS: *Agricultural University of Athens, Athens, Greece*
JUDITH KREYENSCHMIDT: *University of Bonn, Bonn, Germany*
ERIN HEADLEY: *Schreiber Foods, Inc., Green Bay, WI, USA*

Cold chain management has become more and more important in national and international supply chain management strategies. The key objectives of cold chain management are to ensure food safety and quality of perishable food products, minimize food waste and optimize logistic processes to maximize shelf life for consumers. Therefore, understanding the weaknesses in temperature controlled supply chains and their impact on the safety and spoilage of food products as well as the impact on logistic processes including transporting, storage and monitoring is critical. To ensure food safety and quality, the implementation of innovative technologies and methods to monitor quality and safety parameters from production to consumption are of vital importance. This includes methods and models to assess and to prolong shelf life, predictive food safety and risk models, new sensor technologies, and economic and marketing strategies within different industrial sectors or countries to satisfy regulatory or other requirements. The objective

of the symposium is to review weaknesses in international temperature controlled supply chains and to present solutions and models to overcome the different challenges, ensuring food safety and quality and minimizing food waste.

S45 How Do We Measure the Effectiveness of Regulatory Food Safety Programs?

DONALD ZINK: *IEH Laboratories & Consulting Group, Lake Forest Park, WA, USA*
ERNEST JULIAN: *Rhode Island Department of Health, Providence, RI, USA*
ROGER COOK: *New Zealand Food Safety Authority, Wellington, New Zealand*
BETH CANNON: *Steritech, Silverthorne, CO, USA*

Over the last decade, multiple regulatory food safety programs have been implemented by various countries (e.g., FSMA). What metrics will be used to evaluate the effectiveness of these programs once established and operating over a set time period? In the past, programs have been implanted without collecting baseline data prior to implementation; so, an accurate comparison cannot be done. These programs are costly to implement and monitor. Therefore, justification of their use by showing by how much they reduce foodborne illness is warranted. Speakers will describe metrics for measuring the effectiveness of their regulatory food safety programs including FSMA, state and local levels, third party audits, and a New Zealand perspective. However, what are the metrics that can be used to measure positive change in human behavior to prevent and control foodborne disease? In the retail sector, companies audited by third parties often mandate interventions beyond what is required by regulation, allowing a broader scope of issues to be examined. Data from third party audits will be used to compare the effectiveness of various strategies for driving the reduction in the occurrence of food safety risk factors at retail. For example, what types of corrective action requirements are most successful at improving performance? The session will close with an opportunity for audience participation.

S46 Balancing Risks and Benefits in Food Safety

MARCO ZEILMAKER: *RIVM, Bilthoven, Netherlands*
MAARTEN NAUTA: *DTU Food, Søborg, Denmark*
JULIANA RUZANTE: *The Pew Charitable Trusts, Washington, D.C., USA*

Risk-benefit analysis (RBAs) is an emerging topic in the area of food safety. More often food safety authorities, industry, producers and consumers are realizing that a unilateral focus on only risks or only benefits associated with the consumption of food products and diet choices is insufficient. Decisions and policy considerations need to be made balancing the adverse and beneficial health effects for a certain food, food component, or intervention. RBAs are by design multi-disciplinary approaches, bringing together diverse experts such as nutritionists, epidemiologists, modelers, toxicologists and microbiologists in a comprehensive assessment that weighs the potential benefits and risks of the case at hand. In the recent years, frameworks like Benefit–Risk Analysis for Foods or BRAFO, and tools like QALIBRA have been developed, although they haven't been widely used yet. This symposium will hopefully be the first of a series of technical discussions that will resume the debate of RBAs in the area of food safety and propel the field forward. The symposium will present the current state of the art in RBAs, as well as a free online tool, QALIBRA, and how RBAs can be applied to both populations and individuals. A study using multi-criteria decision analysis in RBA will be also presented. To achieve this objective, we propose four presentations of 25 minutes each and a final 20-minute section for questions and answers.

S47 Dilemma in Constructive Use of Risk Assessment in a Variable World: All Microbes are Equal But Some Microbes are More Equal Than Others

HEIDY DEN BESTEN: *Wageningen University, Wageningen, Netherlands*
LEON GORRIS: *Unilever, Vlaardingen, Netherlands*
JANE VAN DOREN: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*

Risk assessment often deals with variability and uncertainty, while food safety management often needs to make discrete decisions. The objective of this symposium is to facilitate connecting probabilistic variability (and uncertainty) in Quantitative Risk Assessment (QRA) on the one side and management need for "discrete" decisions on the other side, for a better understanding of how to manage food safety risks in a variable world. Microbiological criteria, processing targets and limits for CCPs, are examples of "lines in the sand." Decisions from legislation or in standard settings are often discrete. But we live in a variable world: microbiology, food processes, raw materials, humans all are inherently variable. All of these aspects are treated in QRAs, but sometimes this variability, as well as explicit communication of uncertainty, undermines the understanding and the confidence in these analyses and their applications. Making models more "accurate" than reality is simply not possible in a variable world, making them more realistic is possible. Understanding the magnitude and sources of variability and uncertainty can aid in decision making, including selecting the most efficient control measures.

The symposium brings speakers and a panel from academia, industry and government to share the latest developments in QRA, lessons learned and experiences in constructive use of QRA to inform decision making under variability and uncertainty. The symposium includes three presentations and a facilitated panel discussion; in order to bridge the gaps and to make connections between risk assessments and decision makers in government, industry and beyond.

S48 Review of New Risk Factor Studies and Application to Restaurant Inspections in the U.S. and Europe

KEVIN SMITH: *U.S. Food and Drug Administration, College Park, MD, USA*
BARBARA KOWALCYK: *RTI International, Research Triangle Park, NC, USA*
CHRISTOPHE DUFOUR: *Mérieux NutriSciences France, Cergy-Pontoise Cedex, France*
ANN MARIE MCNAMARA: *Jack In the Box, San Diego, CA, USA*

Retail risk factor studies are conducted to measure the occurrence of restaurant practices and people behaviors which contribute to foodborne illness cases and outbreaks in retail food establishments. In 2016, FDA will release their new risk factor study. This new study will compare results from fast food restaurants and casual/fine dining restaurants for the first time. This symposium will discuss results from FDA's study; state risk factor studies; risk factor studies and application to health inspection scores in Europe; and the role of using risk factors in foodservice operations to proactively prevent foodborne outbreaks.

S49 How Safe is Your Infants' Powdered Formula: A Tale of *Cronobacter sakazakii*

ROBERT BUCHANAN: *University of Maryland, College Park, MD, USA*
DILEK HEPERKAN: *Istanbul Technical University, Istanbul, Turkey*
JOSHUA GURTLER: *U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, USA*
JUAN AGUIRRE GARCIA: *Universidad de Chile, Santiago, Chile*

Infant formula is one of the most critical foods for human lives. The risk of powdered infant formula (PIF) contamination may occur at any stage of manufacturing, storage and reconstitution for feeding. The extraordinary resistance mechanism combined with the complex ecology of *Cronobacter sakazakii* make it hard for food safety managers to often safeguard against PIF linked infant fatalities. The food safety concern of PIF is very much evident from the fact that World health Organization (WHO) has its own set of guidelines for safe preparation, storage and handling of powdered infant formula. Due to variability in manufacturing processes and technologies used in the manufacturing of PIF throughout the world, it becomes important to update and share knowledge and science to assure food safety of PIF.

S50 An Overview of Emerging Beverage Process Technologies

CARRIE FERSTL: *Covance Laboratories, Inc., Livermore, CA, USA*
MICHAEL KEMPKES: *Diversified Technologies, Inc., Bedford, MA, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration-IFSH, Bedford Park, IL, USA*

Innovation is a key component in the growth and financial viability of the beverage industry globally. New products and new processing technologies are moving into commercial operations at a tremendously fast pace in recent years. Beverages, such as cold-brewed coffee and high pressure processed coconut water, have enjoyed a marked rate of success. Among emerging technologies, high-pressure processing (HPP) and pulsed electric field (PEF) processing technologies have been identified by a recent survey of food industry professionals (industry, academia and government) as two of the most important developing technologies now and in the next 10 years in both Europe and North America. This accelerated pace of innovation makes it challenging to keep up with the necessary scientific assessment of these products and technologies from a microbiological safety and stability standpoint. Furthermore, long-standing and newly adopted regulations in the United States require careful validation of these technologies as predicated in the globally accepted HACCP approach to food safety. This symposium aims to discuss some of this emerging products and technologies in light of microbial safety and stability concerns as well as presenting strategies to evaluate their safety and comply with pertinent regulations.

S51 An International Perspective on the Development of Targeted Food Safety Education for Vulnerable Populations

ELLEN EVANS: *ZERO2FIVE Food Industry Centre, Cardiff, United Kingdom*
YAOHUA FENG: *University of California - Davis, Davis, CA, USA*
MARK DWORKIN: *University of Illinois at Chicago, Chicago, IL, USA*
JESSIE HUNTER: *University of Idaho, Moscow, ID, USA*

Vulnerable consumers may not be aware of their increased risk for foodborne illness. They may be disproportionately impacted by low health literacy and have limited access or exposure to food safety educational material targeted to their unique medical issues. Most food safety educational programs are developed for the general population and do not address at-risk consumers' specific needs. This symposium will provide the attendees with an international perspective on food safety education for vulnerable populations; including highlighting population specific knowledge gaps and available targeted materials. The symposium is designed to facilitate a dialogue informed by an examination of a heterogeneous group of vulnerable populations from diverse international settings.

First, the findings of a UK project will describe the design, development and evaluation of a consumer orientated food safety intervention for chemotherapy patients and their family caregivers. The acceptability and effectiveness of the intervention were determined with food safety knowledge, attitudes and perceptions of risk, control and responsibility increasing significantly post-intervention. The second presentation will compare three educational approaches that target consumers with diabetes. The findings will include that positive deviance was more effective in knowledge gained and behavior reported than interventions with conventional reading. An epidemiologist in the third presentation will examine food safety knowledge and behavior of persons living with HIV in the U.S., Puerto Rico, and China. Available food safety educational materials designed for persons living with AIDS will be reviewed. The final presentation will examine the unique challenges that educators working with deaf teenagers face, including the need to develop a culturally sensitive education program. This presentation will evaluate deaf teenagers food safety knowledge, attitudes and explore efficient educational approaches to meet their needs.

The session will focus on translating research findings into action and developing efficient, targeted educational programs to address each 'at-risk' group's unique needs.

S52 The Evolution of Food Safety Culture

FRANK YIANNAS: *Walmart, Bentonville, AR, USA*
JOANNE TAYLOR: *TSI, Dubai, United Arab Emirates*
JOANNA GILBERT: *Fonterra, Auckland, New Zealand*

In recent years, use of the term 'food safety culture' has grown significantly by professionals in the industry, academia and regulatory.

Despite its increased usage and growing realization of the role organizational culture plays in influencing human behavior, there remain gaps in a more science-based approach to this topic based on insights from the behavioral science field, concepts of organizational culture, and the use of proven and effective measurement tools.

This symposium is intended to advance the food safety culture discussion from mere awareness exercises to proven strategies used by advanced practitioners on topics related to culture measurements, proven principles of human behavior and real-world industry best practices.

S53 What to Consider When Chemicals Meet Equipment

EDYTA MARGAS: *Buhler, Uzwil, Switzerland*
JOHN HOLAH: *Holchem Laboratories Ltd., Bury, United Kingdom*
GARY LARSEN: *Intralox, LLC, Harahan, LA, USA*
DAVID BLOMQUIST: *Ecolab Inc., St. Paul, MN, USA*

Chemicals are intensively applied in food processing plants to clean and sanitize equipment and environment. If not used properly, chemicals may be incompatible with equipment materials and gradually lead to degradation, discoloration, rusting and even destruction of the equipment. Uses of incompatible chemicals could also harborage and increase the risk of foodborne illness. In this session, we will invite experienced speakers from chemical and equipment suppliers, and food equipment company to discuss the interactions of chemical, soil and equipment; the consequence of incompatibility of chemical and surfaces in reducing equipment life and causing possible food safety hazards; and the development of chemical and equipment compatibility with appropriate concentrations, methods and conditions. This symposium is designed to provide assistance in using chemicals to clean and sanitize equipment for the food safety professionals in sanitation, chemical supplying, equipment design, and food safety and quality.

Symposium

S54 Information and the Creation of Positive Economic Incentives for Food Safety Performance

ROBERT SCHARFF: *The Ohio State University, Columbus, OH, USA*
PATRICIA GRIFFIN: *CDC, Atlanta, GA, USA*
MARK MILLER: *University of Maryland, College Park, MD, USA*
CLARE NARROD: *University of Maryland, College Park, MD, USA*
DENIS STEARNS: *Marler Clark, Seattle, WA, USA*

If consumers had full information about where their food came from and which products were making them ill, there would be substantial incentives for food firms to implement optimal food safety controls. For example, when food is home-grown the farmer's family bears the cost of any foodborne illness and the source of the illness is more likely to be known, leading to ideal food safety choices. Alternatively, in a modern market economy with a global supply chain, the possibility of finding the ultimate source of foodborne illness can be remote, resulting in reduced incentives to provide safe food for each link in the global supply chain. This session explores the critical role that information plays in providing economic incentives to improve performance at each link.

New technologies, new legal/regulatory authority and heightened consumer demand for safer foods are changing the way that food companies view and respond to food safety. As public authorities have developed methods to better link illnesses to foods at the point of sale, the costs (in fines, litigation, and reputation) to associated brands have risen. Some food companies are responding to the challenges and opportunities that better information presents with novel supply chain contracts (such as those encouraged by the Global Food Safety Initiative) that improve traceability and accountability for all actors. Critical to any of these supply chain contracts is the proper identification of hazards, especially for high-risk foods and practices, as well as for the interventions used to control contamination. These data are critical to strengthening economic incentives for adoption of improved, science-based food safety practices; as well as to provide incentives for investment in research and development to invent new and improved food safety systems.

S55 The Use of Whole Genome Sequencing and Metagenomics in Modelling and Risk Assessment

BARBARA KOWALCYK: *RTI International, Research Triangle Park, NC, USA*
CIAN O'MAHONY: *Creme Global, Dublin, Ireland*
LEON GORRIS: *Unilever, Vlaardingen, Netherlands*

In the last number of years, whole genome sequencing and metagenomics have become powerful and important tools in food safety, providing detailed profiling of individual bacterial isolates and the microbiome of various environments and food matrices. However, the significance of how such techniques and data sets can be used in quantitative risk assessment and predictive modeling is less well understood. While there are clearly opportunities for development in this area, a number of challenges exist. These include understanding the role of the microbiome in influencing risk, analysing and managing the volume of data that is generated by such approaches. Furthermore, linking the analysed data in a quantitative sense to appropriate models that can produce output that is of use in decision-making with respect to food safety, particularly in light of a globalized food trade system.

This symposium will present examples of current initiatives that are seeking to bridge this knowledge gap. Case studies will include a project currently underway, in collaboration with a number of large food companies, seeking to develop a monitoring program. The program will track the evolution of the microbiome in different manufacturing facilities and link this to the prevalence of specific pathogens of concern, identified using whole genome sequencing. The need for joined-up and coordinated developments of capabilities and data repositories at the global level is argued. In order to realize the opportunities, WGS approaches offer in international trade. The implications of these new technologies for surveillance and the insights can provide into exposure assessment and hazard characterization will also be discussed.

S56 Whole Genome Sequence Approaches as Applied to *Salmonella:* De Novo Tools for Use in Predictive Microbiology

ROGER LEVESQUE: *University of Laval, Québec, QC, Canada*
LUCAS WIJNANDS: *RIVM - Centre for Infectious Disease Control, Bilthoven, Netherlands*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*

Salmonella enterica is a major cause of global foodborne gastroenteritis, causing millions of cases annually. In the European Union, this pathogen is the leading cause of foodborne outbreaks. In the United States, it is responsible for the most hospitalizations and deaths of any foodborne pathogen. There are more than 2500 serotypes of *S. enterica*, which inhabit a wide range of foods and ecological niches, including low water activity foods that typically do not seem to support the survival of other foodborne pathogens. The diverse nature of *S. enterica* serotypes and its presence in many different foods, makes the use of predictive modelling approaches imperative to define the risk of *Salmonella* contamination of a given food. Whole genomic-based sequencing and gene expression analysis offer an emerging and unparalleled approach to study the emergence and persistence of foodborne pathogens. For example, selectively screening whole microbial genome sequences, and assessing gene expression via epigenetic methods allows for identification of genes with specific functionalities, which can be used to identify potential food safety risks present in a single bacterial isolate or a food sample. The antibiotic resistance profile or virulence potential of a specific bacterium can be investigated by comparing its genome sequence to reference databases containing known resistance genes and virulence factors. Similar approaches have been described to evaluate the persistence of bacteria in food products and resistance to cleaning procedures commonly used in food production settings. Using the example of *S. enterica*, this symposium will highlight the application of whole genome sequencing and gene expression approaches as tools for development of predictive models of *Salmonella* virulence, survival, persistence and growth in foods and the food production environment.

S57 Food Safety Concerns and Testing Challenges in the Emerging Cannabis Products Market

KEITH WARRINER: *University of Guelph, Guelph, ON, Canada*
CHRISTOPHER HUDULLA: *ProVerde Laboratories, Milford, MA, USA*
ROGER BRAUNINGER: *A2LA, Frederick, MD, USA*
ALEXANDRA TUDOR: *TEQ Analytical Labs, Aurora, CO, USA*
CHRIS LINDSAY: *Marijuana Policy Project, Washington, D.C., USA*

The emerging market for edible cannabis products is growing steadily in the U.S., whether for medical or recreational end use. Since 2014, there have been several recalls of cannabis products for reasons of microbial contamination. Other areas for concern include pesticide and allergen residues, environmental monitoring, proficiency testing, method oversight, and inconsistent and varied state regulations. It is incumbent upon food safety professionals and regulators to guide this emerging industry, using good science, proven HACCP approaches and sound analytical methodology. As more states move toward legalization, growers and formulators alike need to be mindful of the microbiological (and chemical) risks associated with scaling

operations and delivering safe product to consumers. The intent of the symposium is to leverage regulatory, commercial, microbiological, and analytical expertise to present the current state-of-affairs in the cannabis industry. The purpose for this symposium is to begin a broad discussion on the current science of cannabis products, regulatory framework, current microbiological and chemical methods utilized, sampling protocols, manufacturing challenges and need for standardization/proficiency management within the context of food safety. IAFP has several potential roles to play in this emerging marketing, the least of which is bringing sound microbiological and allergen science to regulators setting specifications for finished product testing.

[For the first time in conference history, AOAC held a 90-minute introductory symposium on the topic of cannabis testing methodology at the 2015 conference in Los Angeles. Unfortunately, the topic of product safety was not addressed during the meeting. The symposium proposed here for IAFP 2016 would be a logical continuation on what was discussed at AOAC 2015.]

S58 FDA Food Safety Modernization Act (FSMA) and Small Processors: Identifying Challenges and Addressing Concerns

PURNENDU VASAVADA: *PCV & Associates, LLC, River Falls, WI, USA*
CRAIG HENRY: *Decernis, Washington, D.C., USA*
RAM RAO: *U.S. Department of Agriculture, Washington, D.C., USA*
BRUCE FERREE: *California Natural Products, Lathrop, CA, USA*

Food Safety Modernization Act (FSMA) was signed into law in 2011. Implementing the most comprehensive reform of this nation's food safety system in more than 70 years, requires robust strategies. FDA has released Final Rules for the Preventive Controls for Human Food and Preventive Controls for Animal Food. Small processors are facing challenges, including understanding the nuances of the new regulations and FDA expectations. The FSMA emphasizes public-private partnership and the FDA has funded the Food Safety Preventive Controls Alliance (FSPC) to provide educational training and outreach to small processors. This session will discuss challenges and opportunities as well as the FSPC standardized training and outreach program designed to help implementation of FSMA.

S59 FSMA and ISO 17025 Accreditation in a Food Testing Laboratory

BRAD GOSKOWICZ: *Microbiologics, St. Cloud, MN, USA*
HEATHER JORDAN: *American Proficiency Institute, Traverse City, MI, USA*
BRADLEY STAWICK: *Microbac Laboratories, Bartlett, TN, USA*
REAGAN CONVERSE: *North Carolina Department of Agriculture and Consumer Services, Raleigh, NC, USA*

With passage of the Food Safety Modernization Act, the FDA will require test results for certain categories of food imports to be obtained from ISO 17025 accredited laboratories. While old news to much of the world, this is a significant change in the United States, where until FSMA was drafted, accredited laboratory testing was not an FDA requirement. This stance of the FDA will help push accreditation towards it being a baseline to demonstrate competence to deliver reliable and accurate results.

Associated with this approach, are a number of related factors linked to accreditation that now must be considered by the food laboratory testing industry. These are the need for the laboratory to utilize consistent and traceable reference materials and demonstration of proficiency in the testing activities to be included on their Scope of Accreditation. In food microbiology this usually means the use of certified reference cultures and recognized proficiency testing providers. A brief overview of the accrediting process will set the stage for the speakers: 1) the background and quality control activities involved in being a reference material producer, 2) the role that proficiency testing programs and schemes provide in demonstrating that the laboratory can produce good, reliable data; 3) how the implementation of these requirements impacts in a multi-site contract testing laboratory; and 4) a perspective of FSMA's impact from a state testing laboratory.

S60 Lab Detection of Food Safety Hazards: Has Sample Prep Advanced into the 21st Century?

THOMAS TAYLOR: *Texas A&M University, College Station, TX, USA*
EFSTATHIA PAPAFRAGKOU: *U.S. Food and Drug Administration-CFSAN, Laurel, MD, USA*
SANDRA TALLENT: *U.S. Food and Drug Administration, College Park, MD, USA*
SUSAN LEONARD: *U.S. Food and Drug Administration, Laurel, MD, USA*

As detection technologies have moved into the 21st Century to improve the sensitivity, specificity, and speed of food product analysis, including methods employing whole genome sequencing, metagenomics and mass spectrometry, the rate-limiting step for start-to-finish processing remains sample preparation. The challenges include several key points: (1) is the target a pathogen (biotic) or chemical/toxin (abiotic), (2) is there bias in the extraction process, and (3) does the recovery efficiency provide a sufficient level of target for downstream analysis, considering the sensitivity of the detection method? In an ideal world, live pathogens would be enriched/cultured in one "universal" medium, isolated and characterized within one working day in the analytical laboratory. For microbes or chemicals/toxins that cannot be amplified by culture means, extraction processes would be designed to isolate a sufficient amount of material for further identification in assays that are based on small sample volumes. This symposium will address these most relevant issues, current status with respect to several pathogens or toxins/chemicals, and how current state-of-the-art technology is dependent upon sample preparation and efficient extraction methods.

S61 Nanophysical, Electrical and Chemical Biology Approaches for Control of Bacterial Biofilms

CARMEN MORARU: *Cornell University, Ithaca, NY, USA*
HERMAN SINTIM: *Purdue University, West Lafayette, IN, USA*
ROBIN PATEL: *Mayo Clinic, Rochester, MN, USA*

Biofilms, the predominant microbial phenotype in nature, present special problems to the food and allied industries. For example, they exhibit reduced sensitivities to traditional means for inactivation, such as physical processes, sanitizers, antimicrobials or antibiotics. Biofilms may also serve as sources for continual shedding of microbial cells, leading to recontamination of equipment and foods processed thereon. Strategies for effective control of biofilms would be of wide benefit to not only the food industry and other sectors of agriculture, but also to the healthcare, water processing, shipping and other industries. This mini-symposium will present three promising strategies for control of biofilms, wherever they may occur. These include nanoscale surface engineering, chemical biological and electrical approaches for reducing microbial attachment to surfaces and inhibiting subsequent biofilm formation. The engineering- and chemistry-based approaches discussed here may have ready applications to control of biofilms in various industrial settings, including the food industry.

S62 Building and Sustaining Support for Your Food Safety System: How to Communicate with Senior Management, Production Line Operators, and All Levels in Between

MIKE BOLAND: *University of Minnesota, Minneapolis, MN, USA*
SHAWN STEVENS: *Food Industry Counsel LLC, Milwaukee, WI, USA*
DONNA BEEGLE: *Communication Barriers, Portland, OR, USA*

This symposium will explore strategies that food safety professionals can use to build and sustain support for food safety programs within their organizations. Topic areas that will be covered include building a financial case for food safety, meaningful messaging for food industry executives to all tiers of employees, legal perspective of food safety, as well as successful initiatives from within the food industry. The session will include speakers from various backgrounds, such as a Food Safety Litigator, a business professor, and a socio-economic communications expert. This cross section of viewpoints from outside the food safety profession will provide the audience an "outside-in" look at how food safety messages can have the most immediate and long-lasting impact. The session will conclude with all speakers available for questions and final thoughts for building and sustaining support for food safety programs. Given the breadth of material and diversity of speakers, we are recommending this session to be a 1.5 hr symposium.

S63 Antimicrobial Food Packaging: Breakthroughs and Benefits That Impact Food Safety

CYNTHIA EBNER: *Sealed Air, Duncan, SC, USA*
DEVON HILL: *Keller and Heckman LLP, Washington, D.C., USA*
KAY COOKSEY: *Clemson University, Clemson, SC, USA*

Packaging plays a critical role in food safety and quality. Packaging prevents contamination of food from pathogens, spoilage organisms, chemical and physical hazards and intentional tampering. It also provides an opportunity and platform to convey important information to consumers. Beyond food safety and quality, pertinent information such as safe handling and cooking procedures, sell by or use by dates, and ingredient lists can all be delivered as additional important benefits. Of special note, packaging can control the atmosphere that is present inside of the package and so inhibit unwanted microbial growth. Packages can even function as cooking vessels for food and thus minimize the handling of the food during preparation. Clearly, food packaging does a lot to protect the global food supply, but can it do more? One concept that has been studied extensively is the idea of packaging that exerts an active antimicrobial effect on organisms that may be present on the food inside the package via incorporation of antimicrobial agents or processes into the package itself. This symposium will showcase antimicrobial packaging and some of the difficulties encountered when attempting to exert an antimicrobial effect on the entirety of a packaged food via the package itself. The regulations that pertain to such processes will be presented, as will the latest technologies for incorporating antimicrobial agents into the package. Finally, technologies that may be able to provide a non-thermal antimicrobial process through a sealed package will be highlighted.

S64 Close Call: Assessing Risks of Food Packaging That Can Impact Food Safety

CHENG-AN HWANG: *U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, USA*
YOONSEOK SONG: *U.S. Food and Drug Administration-IFSH, Bedford Park, IL, USA*
CIAN O'MAHONY: *Creme Global, Dublin, Ireland*

In the diverse and multi-faceted global processed food industry, packaging is the quintessential preventive control. Food packaging is designed to protect the food from physical damage during transportation. It also serves as an aegis against product contamination for the entire shelf life of the food while it is in commerce. Regulatory agencies have long recognized the important function of food packaging in preserving the public health status of the food. Clearly, this is a very complicated issue and there are myriad of challenges associated with selecting and qualifying packaging for the many varied food forms that are offered for sale in the global marketplace. Functionality of food packing is inextricably correlated with the nature of the food preservation and processing methods, the composition of the foodstuff and the food's intended method of delivery. Further complicating the situation are the chemical and physical properties of the packaging materials. For example composite packaging may contain structural and functional polymers, colorants, as well as adhesives, ink and other chemicals used for controlling barrier and structural properties. Understanding these components and their potential implications for the food they will contain should be assessed and validated as a function of the Hazard Analysis and Preventive Control process. No matter the packaging format, be it glass bottles, metal cans, flexible pouches or foil wrappers, each has inherent risks. Failure Modes and Effects Analysis and Probabilistic Exposure Modelling are effective risk assessment strategies. Achieving science based results from the risk assessment process requires involvement of a cross functional team that might include packaging engineers, process engineers, product developers, toxicologists, regulatory specialists, physical chemists, sensory experts and food safety personnel. This symposium will offer critical insights into the processes involved with assessing risks that may be associated with food packaging.

S65 Food Safety Challenges and Issues in India in Context of New Food Safety Regulations and the US FSMA

PURNENDU VASAVADA: *PCV & Associates, LLC, River Falls, WI, USA*
JENNY SCOTT: *U.S. Food and Drug Administration, College Park, MD, USA*
NILESH AMRITKAR: *Envirocare Labs, Thane, India*
ASHOK VASUDEVAN: *Preferred Foods International, Stamford, CT, USA*

Food processing industry is one of the largest industries and a prominent sector of Indian economy. Food is the biggest consumption category in India with 31% of the consumer's expenditure. The Indian food industry is expected to reach Euro 181 billion in 2015 and Euro 223 billion by 2020. In recent decades, the Indian food industry has increasingly adopted internationally recognized food safety and quality systems such as the HACCP and GFSI programs such as the SQF and BRC. Recognizing the significance of food safety and quality for domestic and export market, the Indian government has initiated extensive reforms, including implementation of the Food Standard and Security Act of India (FSSAI). However, Indian food industry is facing several food safety issues and challenges as evidenced by well publicized incidences of recalls and illnesses resulting from contamination and food adulteration. The Food Safety Modernization Act (FSMA) when finalized and implemented is expected to bring a sea change of food protection standards not only in the U.S. but also in India and other countries involved in food trade with the USA. Per FSMA, countries exporting human food and animal feed will have to implement new and enhanced food protection standards for risk-based hazard analysis and preventive controls, produce safety, the Foreign Supplier Verification Program (FSVP), and other related rules. There are significant challenges and issues involved in understanding and complying with the FSMA requirements. This symposium is designed to explore the current status, challenges and issues in food safety and impact of FSMA on international trade between the US and India. Speakers from food industry, government agencies, and academia from India and the USA will provide unique perspective on contemporary food safety issues and challenges and impact of FSMA on international trade between the US and India.

S66 Disinfectant By-products in Wash Water, Vegetables and Fruits

ALEXANDER LEMKE: *Chemisches und Veterinäruntersuchungsamt Stuttgart, Württemberg, Germany*
RICHARD WEISMAN: *US EPA, Washington, D.C., USA*
DAVID SMITH: *U.S. Department of Agriculture-ARS, Animal Metabolism-Agricultural Chemicals Research, Fargo, ND, USA*
XUETONG FAN: *U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, USA*

Disinfectants (sanitizers) are often used by the produce industry in the wash water to reduce the population of microorganisms in the products/water and to minimize cross contamination. Washing with sanitizers is one of the critical processing steps for fresh and fresh-cut produce. The most common sanitizers used by the fresh produce industry in the U.S. are chlorine-based compounds, such as sodium hypochlorite (chlorine) and chlorine dioxide. However, there is a concern about the use of chlorine in the fresh produce industry and other industries due to potential environmental and health risks associated with the formation of carcinogenic halogenated disinfection by-products (DBPs). Chlorine reacts with organic matter and forms carcinogenic halogenated DBPs. Partly due to the possible generation of halogenated DBPs in the water, the use of chlorine in fresh-cut produce washing is prohibited in European countries. A recent survey of fruits and vegetables in Europe suggested that perchlorate and chlorate are present in fruits and vegetables at levels exceeding the maximum residue limit of 0.01 mg/kg imposed by the European Union. It is suspected that chlorination of water may contribute to chlorate and perchlorate formation. The symposium will present the most updated information on formation of DBPs, factors contributing to the formations, means to minimize the accumulations in water and produce, detection methods, the risk of BBPs for human health, and regulatory aspects.

S67 Integrating Food Safety into Food Security

MARY KENNY: *Food and Agriculture Organization, Rome, Italy*
DAVID CREAN: *Mars Inc., Mclean, VA, USA*
RUTH ONIANG'O: *Rural Outreach Program (ROP) Africa, Nairobi, Kenya*

About 2 million people die annually from contaminated food and water, typically in areas which suffer from food insecurity. Aflatoxin may cause 30% of liver cancer cases globally each year from spoiled crops. Around 100 million tons of food are wasted annually in the EU, enough to feed the hungry in the world two times over. The "four pillars" of food security are the availability of food, access to populations, the stability of food sources over time and utilization. Utilization includes metabolism/nutrition as well as food safety and sanitation. Food safety can be impacted by ways of processing and preparing of food, linked to sanitation that can determine the likelihood of contamination sources. Associated with food safety is adequate health care and education for individuals to make informed safe food choices. However, food safety has rarely been a priority in international discussions on food security, partly because the disciplines involved in policy discussion do not always intersect. The FAO has highlighted the need for policies and strategies to ensure not only adequate food but safe food and is collaborating with the organizers to formulate approaches to the symposium. Speakers will address the global situation from UN and industry perspectives, as well as a more detailed discussion on issues facing Africa, and allow for audience interaction to a panel. Addressing food safety to achieve food and nutrition security should lead to the improved safety of foods in domestic supply chains, and the safety of foods distributed through emergency food aid programs.

S68 Approaches to Safe Use of Irrigation and Wash Water in the Face of Increased Global Water Shortages

SURESH D. PILLAI: *Texas A&M University, College Station, TX, USA*
MANAN SHARMA: *U.S. Department of Agriculture ARS EMFSL, Beltsville, MD, USA*
EWEN TODD: *American University of Beirut and Ewen Todd Consulting, Okemos, MI, USA*
OSAMA EL-TAWIL: *Cairo University, Cairo, Egypt*

Increasing water availability and sustaining water supplies are of strategic importance for food production globally. This symposium will focus on the issue of limited safe irrigation and wash water in the face of potable water shortages and will present possible mitigation strategies. Untreated sewage water or clean well-water pumped to surface reservoirs becoming contaminated is used in many countries to irrigate crops and wash produce with risks of infectious and chronic diseases from pathogens and toxic chemicals arising from urban effluent and agricultural and industrial use. Overusing aquifer supplies and climate change on reducing water availability and creating more frequent conditions for drought are already exacerbating existing problems but there is little political will for long-term mitigation strategies. Options such as desalination, reverse osmosis and recycled/reclaimed water to facilitate salt removal and cleanup of wastewater using physical, chemical or biological means will be discussed. Speakers will address the issues from the perspectives of developed and developing countries and regions, e.g., California and Arizona in the U.S., as well as in The Middle East and Africa which have considerable experience with growing crops with limited water supplies in the face of continued drought conditions. There will be time for audience input at the end of the symposium.

S69 Hygienic Design – Cost of Ownership (My Budget Will Not Cover Hygienic Design Expenses)

ALLEN SAYLER: *Center for Food Safety & Regulatory Solutions (CFSRS), Woodbridge, VA, USA*
KNUTH LORENZEN: *EHEDG, Wulfsen, Germany*
JAIME VACA: *Hershey, Hershey, PA, USA*
MICHELLE EVANS: *Diamond Pet Foods, Topeka, KS, USA*

Food processing equipment requires large investments from food processors, especially equipment with higher hygienic design standards as it needs suitable construction materials and often more advanced manufacturing methods. At the same time, there is constant pressure to lower food prices, making it difficult to justify purchasing equipment with a higher price. The important factor, therefore, is to understand the total cost of ownership of hygienically designed equipment throughout the whole lifecycle of the machine. For food safety experts, the largest benefit of hygienically designed equipment is a reduction of hazards, such as the growth of microorganisms or introduction of contaminants. The procurement personnel, however, often do not understand how large the risk is associated with the equipment and how it translates to money. Some prefer to take the risk and assume that all incidents happen elsewhere, not in their own business. This seminar is designed to bridge the gap between engineers, procurement and food safety experts in food companies. The main objectives of the program are to demonstrate how to relate hygienic design features of the equipment to tangible benefits such as reduction of cleaning time, faster changeovers, the extension of shelf life of food, extension of the lifecycle of the equipment, successful audits, etc. Different methods of quantifying the hygienic design benefits and case studies from the food and pet food industry, regulatory bodies and standards providers will be presented.

S70 2016 Foodborne Outbreak Updates

CARLOTA MEDUS: *Minnesota Department of Health, St. Paul, MN, USA*
JENNIFER SINATRA: *U.S. Department of Agriculture-FSIS, Washington, D.C., USA*
ELIZABETH SZABO: *NSW Food Authority, Silverwater, New South Wales, Australia*

Introduction: There are many lessons to be learned from reviewing the results of foodborne outbreak investigations that can direct future investigations and potentially identify factors leading to mitigation strategies that can be applied to prevent future illness.

Purpose: This symposium will cover three or four significant foodborne disease outbreaks reported since the 2015 IAFP Annual Meeting.

Methods: We are seeking to have two outbreaks from North American (Bonnie Kissler, CDC), another from a developed country outside North America and possibly one from a deve oping country. Appropriate speakers to address the international outbreaks will be identified closer to the meeting date. Following the presentations, an opportunity would be given for the audience to provide information on outbreaks that have occurred in their jurisdictions in the past year.

Results: Details of four recent foodborne outbreaks will be presented with steps of the investigations outlined and lessons to be learned.

Significance: With the globalization of our food supply, outbreaks can result in very large case numbers. Frequently new pathogen and food vehicles are identified. If we are to prevent future cases of foodborne disease, industry, regulators and food safety scientists need to be aware of the circumstances that lead to these outbreaks and their causation.

S71 FSMA Preventive Controls for Produce Packing and Cooling Operations: A Reality Check and Near-term Aspirational Compliance Roadmap

ANNEMARIE BUCHHOLZ: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
TREVOR SUSLOW: *University of California-Davis, Davis, CA, USA*
MICHELLE DANYLUK: *University of Florida, Lake Alfred, FL, USA*
JOSEPH STOUT: *Commercial Food Sanitation, LLC, Libertyville, IL, USA*

With the release of several key final rules under FSMA, a significant proportion of the fresh produce industry responsible for handling and shipping Raw Agricultural Commodities (RACs), will not 'qualify' as 'primary activities farms' or 'secondary activities farms', as defined in the Preventive Controls Rule. As a consequence, many of these facilities will be challenged to make unprecedented infrastructural and cultural changes to meet applicable compliance expectations. A food safety systems approach to proactively manage compliance may align with directives within FSMA or customer requirements. Currently, customer requirements are taking a broader scope of inclusivity among packing operations and a prescriptive approach to certain standards, critical limits, and testing/monitoring programs. Fundamental to culture change is recognizing that RAC is barrier term to progress and all packing operations should refashion themselves as food handlers. Whether legally or market-reality bound to implement Preventive Controls, many handlers and shippers of RACs are currently operating significantly outside the parameters of design and management expectations for optimal cleaning and sanitation. Sanitary equipment design and fabrication of surfaces would not currently meet the criteria for FSMA-PC compliance. For most operations, but especially midsize and smaller packers, the cost of retrofitting or replacing equipment and related facility functional zones to achieve risk reduction and preventive control programs is economically burdensome. This mini-symposium will review key observations from the outbreak and associated environmental investigations at RAC packing operations and current research-based surveys and environmental monitoring data from a range of small-scale to large shipping-point facilities. Within the context of an aspirational roadmap and realistic timeline, prioritized implementation goals for the industry and the research and development community will be presented for participatory session discussion.

S72 Debate: Raw Milk Sales and Consumption – An Amicable Exchange of Experts

JOSEPH HECKMAN: *Rutgers University, New Brunswick, NJ, USA*
JEFF KORNACKI: *Kornacki Microbiology Solutions, Inc., McFarland, WI, USA*
JEFFREY FARBER: *University of Guelph, Guelph, ON, Canada*
THEODORE BEALS: •*Board Member, Farm to Consumer Foundation, Grass Lakes, MI, USA*

Raw milk advocates argue that its consumption has health benefits, not found in pasteurized milk. They believe that these benefits outweigh any food safety risks. Many in the food safety community, however, believe that unpasteurized milk is a high-risk food product and its consumption should not be promoted, as its dangers outweigh any alleged benefits. In this debate, experts from the pro-raw milk and the pro-pasteurization sides will square off, in a highly controlled and moderated format, for a risk-benefit analysis of raw milk consumption. A previous debate on this topic, held at Harvard University, in 2012, included no Ph.D. or M.D.-credentialed participants. This exchange will include 3 Ph.D. and 1 M.D./M.S.-degreed professionals. Video-recording this exchange will provide a highly useful tool for reaching out to individuals on both sides of this issue, and will encourage thoughtful consideration of opposition arguments.

S73 Revisiting the STEC Testing Approach: Regulatory and Industry Perspectives on Making It More Reliable for Routine Application in Food Industry

EMILIO ESTEBAN: *U.S. Department of Agriculture-FSIS-OPHS-EALS, Athens, GA, USA*
BETSY BOOREN: *American Meat Institute Foundation, Washington, D.C., USA*
PETER FENG: *U.S. Food and Drug Administration, College Park, MD, USA*
PATRICK FACH: *ANSES, Paris, France*

Out of few hundreds Shiga toxin producing *E. coli* serotypes (STEC), only a subset causes human illnesses. Agencies such as FSIS currently regulates O26, O111, O103, O121, O145 and O45 along with O157: H7 (so called Top-7) as adulterants in beef trim. Thus, a very specific testing approach based on genetic markers (virulence factors) to assure the confirmation of these top 5–7 STEC is needed. Unfortunately, the current methodologies (based on Shiga toxins and Intimin genes) end up in high numbers of false positives at different stages of confirmation (Potential-Presumptive-Confirmed positives) requiring almost 57 days. The risk assessment based on current testing methods has become questionable both economically and scientifically specifically when the shelf-life of food products such as meat & produce products is very short.

In addition to intimin/ Shiga toxin, there are other recognized, and likely unrecognized, additional virulence determinants required for reliable detection and confirmation of pathogenic STEC. The FDA, CDC, FSIS, and other agencies believe that enhancing the scientific information available on STECs and improved detection and identification methodologies will result in reduced STEC illnesses. Therefore, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) subcommittee was charged to identify virulence factors and attributes that define foodborne STEC as severe human pathogens.

In this session, the world renowned STEC experts will evaluate the current state of STEC testing of U.S. beef products from regulatory and industry perspectives. Further, the NACMCF sub-committee objectives will be described in addressing current STEC testing issues. New approaches for STEC testing based on virulence factors along with a future roadmap will be discussed.

The talks will be concluded with an interactive panel discussion ensuring participation from audience geared towards 'if it should be a testing for pathogenic *E. coli* or just Top-7'. A one-page summary of the session will be published as a white paper in *Food Protection Trends*.

S74 We are What We Eat: Should Food Microbiology Take the Lead on Understanding How the Homeostasis of the Gut Microbiome Influences Human Health and Disease?

LAURA KAHN: *Princeton University, Princeton, NJ, USA*
ERIC MARTENS: *University of Michigan Medical School, Ann Arbor, MI, USA*
PATRICIA HIBBERD: *Harvard University, Boston, MA, USA*

The One Health Initiative is a global health care strategy for expanding interdisciplinary collaborations and communications in all aspects of human health in relation to the intersections among humans, food animals and the environment including farm and food manufacturing arenas. A number of studies suggest that obesity, glucose homeostasis disorders (e.g., type 2 diabetes, impaired fasting glucose, glucose intolerance, and insulin resistance), lipid homeostasis disorders (i.e., dyslipidemia) and cardiovascular diseases (e.g., hypertension and fibrolysis disorders) are associated with profound metabolic imbalances, all related to the role of diet and the interaction of an individual's gut microbiome. As an example, lactate is one nutrient that can be utilized by certain Firmicutes–members of the gut microbiome–to form fatty acids, such as butyrate, acetate, and propionate, and may be important for maintaining a stable gut microbial community. Many of the lactic acid bacteria are considered to be probiotics; and in 2011, $28 billion was spent in the U.S. on probiotic foods (particularly yogurt) and prebiotic supplements. Monitoring food composition and nutrient intake by consumers are foundational to promulgate public policy. This symposium brings together speakers who will discuss the relationship of the "One Health Initiative" and food microbiology to address the connections between the environment, food animals, and their effects on human health. Specific topics include the safety of prebiotics, probiotics, antibiotics, and other supplements, e.g., non-caloric artificial sweetener formulations in our diet and their effect on the metabolic activity of the gut microbiome to influence an individual's overall health. IAFP members will gain a better understanding of the current status of dietary components, antibiotics and nutritional supplements which compose our and diet and how this diet modulates the gut microbiota composition, and how an altered microbiota can exert profound effects on host physiology and metabolism.

S75 The Global Burden of Foodborne Disease

BRECHT DEVLEESSCHAUWER: *Wetenschappelijk Instituut Volksgezondheid, Brussels, Belgium*
ARIE HAVELAAR: *University of Florida, Gainesville, FL, USA*
EMILIO ESTEBAN: *U.S. Department of Agriculture-FSIS-OPHS-EALS, Athens, GA, USA*

This symposium will present and discuss the key findings of the WHO Initiative to Estimate the Global Burden of Foodborne Diseases, which for the first time presents an estimate of the global burden of foodborne disease. Illness and death from diseases caused by unsafe food are a constant threat to public health security, as well as socio-economic development throughout the world. A wide diversity of hazards, including viral, bacterial, parasitic and chemical, can be transmitted by food causing a broad range of diseases, ranging from acute to chronic and some with very high case-fatality ratios. The WHO Initiative to Estimate the Global Burden of Foodborne Diseases was launched by the World Health Organization (WHO) and the Initiative was supported by the Foodborne Disease Burden Epidemiology Reference group (FERG). FERG estimates are based on a burden of disease approach, i.e., using a summary measure of population health to assess and compare the relative impact of different diseases and injuries on populations. Using Disability Adjusted Life Years (DALYs), FERG quantified global and regional health losses due to illness and death caused by foodborne hazards. A major achievement is that estimates are not only made in regions where more and better data are available, but also in all other regions of the world, making the best use of available data.

S76 Strategies to Identify Foodborne Parasites: A Global Perspective toward Improving the Safety of Food Supply

TBD TBD: *TBD, TBD, IA, USA*
MOMAR NDAO: *McGill University, Montreal, QC, Canada*
ALEXANDRE DASILVA: *U.S. Food and Drug Administration, Laurel, MD, USA*
ALEXANDRE D. T. COSTA: *Fiocruz, Curitiba, Brazil*

This symposium will describe the latest efforts by academia, industry, public health agencies in the U.S. and abroad on test development and NGS approaches to detect and characterize foodborne parasites on a variety of samples. The speakers, representing institutions from North America, Europe and South America, will describe recent efforts in developing advanced strategies for foodborne parasites based on both conventional and next generation sequencing methods. The main objective of the symposium is to highlight current accomplishments, as well as, future opportunities and challenges to control, minimize or eliminate the presence of foodborne parasites in different food commodities. Due to the globalization of foods, extensive international collaborative efforts are needed to harmonize strategies that can improve the food safety at a global level. This symposium will target laboratory scientists, epidemiologists and regulators interested in knowing more about the global efforts in foodborne parasitology.

Roundtable Abstracts

RT1 A Real-world Conversation about Food Safety and Microbial Quality of Sustainable Diversified Farming Systems

SAMIR ASSAR: *U.S. Food and Drug Administration, College Park, MD, USA*
JAMES GORNY: *PMA, Davis, CA, USA*
TREVOR SUSLOW: *University of California-Davis, Davis, CA, USA*
STEVE WARSHAWE: *Beneficial Farms CSA, Sante Fe, NM, USA*
KAREN MCSWAIN: *Carolina Farm Stewardship Association, Pittsboro, NC, USA*

Symposia afford attendees the opportunity to hear the latest information on a specific topic with little time for questions. For emerging and controversial topics it affords little or no time for adequate question and answer. The purpose of this roundtable is to facilitate discussion on the Safety and Microbial Quality of Foods produced at the fresh produce-animal interface in diversified sustainable farms that cater to the local community. This roundtable will address the following topics: 1- Define what is a diversified farm within our national context, 2- Explore production practices within these systems, 3- Describe the potential food safety risks associated with these management practices, 4- Describe the challenges of developing holistic, systems-based approaches to developing a body of scientific knowledge specific to fresh produce safety and these farming systems, 5- Frame current FSMA regulations and their potential implications for diversified farms 6- How produce associations are organizing and supporting their growers with the implementation of these new mandates, 7- Scientific approaches that will address the needs of this industry during rule enforcement.

RT2 The Global Food Safety Kaleidoscope: A Look at Food Safety Priorities through Various Cultural Lenses

JEAN KAMANZI: *The World Bank, Washington, D.C., USA*
ANDREW CLARKE: *SAI Global, Toronto, ON, Canada*
BOBBY KRISHNA: *Dubai Municipality, Dubai, United Arab Emirates*
PAUL VILCHES: *Hershey's, Guadalajara, Mexico*
NATALIE DYENSON: *Walmart, Fayetteville, AR, USA*

Food safety in the Western world has historically focused on areas such as foodborne pathogens and allergens; however, the rest of the world has additional concerns. Some include the availability of food, economic adulteration, polluted water supplies, etc. The assessment of risk is hugely impacted by having enough food to feed a population. This disconnect within the food trading countries is an interesting area to reflect upon, especially now as we move into the FSMA era.

The objective of the proposed roundtable is to understand philosophies and the focus that different regions place on food safety in their food systems and culture. Some of the topics of discussion will include:

- The hazards of concern and typical control measures (including GMPs) in various regions of the world
- The prevalence of food fraud in the global supply chain
- To what extent do national/regional/local cultural differences affect food safety systems and decisions?
- How do locally based auditors help or hinder (influence) the development of common standards, such as GFSI?
- The differing approach to governmental regulations and enforcement
- To what extent do judicial and liability concerns affect the food industry in your area of the world?
- How are different regions preparing for FSMA Foreign Supplier Verification rule and do all countries know (or care) about it?
- How does the transparency and the complexity of supply chains vary by region?

In a truly international fashion, this roundtable will raise awareness among the food industry about the food safety perspectives of various countries/regions of the world. It will initiate dialogue and create collaborations to strengthen food safety.

RT3 Undesirable Microorganisms – Determining When Food Spoilage becomes Food Safety, and When It Does Not

MICKEY PARISH: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
WILLIAM SHAW: *U.S. Department of Agriculture-FSIS, Washington, D.C., USA*
MELINDA HAYMAN: *Grocery Manufacturers Association, Washington, D.C., USA*
RUTH PETRAN: *Ecolab, Inc., Eagan, MN, USA*

Microbial spoilage of food and beverage products can be caused by a number of factors, such as a loss of process control, post-processing contamination, inadequate packaging performance, damage during distribution, or temperature abuse during storage or display. The causative microorganisms of food and beverage spoilage are usually not the same as those that are attributable to foodborne illness. However, in some instances in North America, spoiled products have been subjected to class II recalls, rather than a more discreet market withdrawal. With the recent mandatory recall authorization provided to the U.S. Food and Drug Administration in the Food Safety Modernization Act, the difference between "market withdrawal" and "recall" is an important regulatory and legal matter that impacts food brands and the bottom line for food businesses.

The term "undesirable microorganisms" as defined in the Hazard Analysis and Risk-based Preventive Control Final Rule includes those microorganisms that are of public health significance, that subject food to decomposition, that indicate that food is contaminated with filth, or that otherwise may cause food to be adulterated. However, questions remain as to what circumstances and which microorganism-product interactions turn a food spoilage event into a food safety concern. Consumers are also confused about the differences between true food safety hazards and undesirable, but innocuous, food spoilage. Expert interpretation of the microbiological data, risk assessments on the product, product status in the marketplace, and normal consumer use and handling of the product must be evaluated on a case-by-case basis to make these decisions. This roundtable will focus on the specific microorganism, product associations and discuss scenarios when food spoilage is a food safety hazard as well as scenarios when there is no food safety concern with spoilage.

RT4 Food Microbiomes: So We Found a Sequence…Big Deal, Now What?

PALMER ORLANDI: *U.S. Food and Drug Administration-CFSAN, Silver Spring, MD, USA*
EDWARD DUDLEY: *The Pennsylvania State University, University Park, PA, USA*
KENDRA NIGHTINGALE: *Texas Tech University, Lubbock, TX, USA*
ERIC BROWN: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
JAMES KAUFMAN: *IBM Almaden Research Center, San Jose, CA, USA*

We are in a new era of studying the microbial ecology of foods. Last year we heard several talks on food microbiomes and learned how the microbial taxa in your products can be determined using gene sequencing and presented in a pie chart or bar graph format all without the need for culture. Yet, as we begin this journey and use this powerful tool, even more, we must begin to ask some obvious questions important to the practicing food microbiologist. Questions such as what is the real meaning of finding DNA sequences of bacterial or fungal taxa in an extract of food? Does this data provide a grounds to take corrective actions should a pathogen sequence wind up in a sample community description? What if that sequence was derived from a non-viable cell? These and other questions you pose will be explored by a roundtable of experts in the techniques of microbiome analysis. The format is to be as interactive and free form as possible so that the audience is allowed a high degree of participation.

RT5 A Debate: Current Perspectives in Food Safety

JEFFREY LEJEUNE: *The Ohio State University, Wooster, OH, USA*
KELLY STEVENS: *General Mills, Inc., Golden Valley, MN, USA*
JOSEPH STOUT: *Commercial Food Sanitation, LLC, Libertyville, IL, USA*
MICHAEL HOLSAPPLE: *Michigan State University, East Lansing, OH, USA*
KATHERINE MJ SWANSON: *KMJ Swanson Food Safety, Inc., Mendota Heights, MN, USA*
BENJAMIN CHAPMAN: *North Carolina State University, Raleigh, NC, USA*

Sponsored by the ILSI North America Technical Committee on Food Microbiology:

This session is dedicated to the memory of John Cerveny.

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: "Is our food supply too clean? Are there unintended health consequences as we chase zero?"; "Does the food industry need to change its approach to food safety management, taking into account cultural differences between boomers and millennials?"; and, "Is there a human health risk introduced from the use of chemical sanitizing agents in food manufacturing that exceeds the benefit from the reduction of microbial risk that is achieved?" Each topic will include a 7-minute presentation in support of (YES) followed by a 7-minute presentation in opposition of (NO) 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.

1. **Is Our Food Supply Too Clean? Are There Unintended Health Consequences as We Chase Zero?**
   JEFF LEJEUNE, *Ohio State University*
   JOE STOUT, *Commercial Food Sanitation, LLC*
2. **Does the Food Industry Need to Change Its Approach to Food Safety Management, Taking into Account Cultural Differences between Boomers and Millennials?**
   BENJAMIN CHAPMAN, *North Carolina State University*
   KELLY STEVENS, *General Mills Inc.*
3. **Is There a Human Health Risk Introduced from the Use of Chemical Sanitizing Agents in Food Manufacturing That Exceeds the Benefit from the Reduction of Microbial Risk That is Achieved?**
   MICHAEL HOLSAPPLE, *Michigan State University*
   KATHERINE MJ SWANSON, *KMJ Swanson Food Safety, Inc.*

RT6 How to Fix Food Safety Education and Enhance Training Effectiveness

MICHAEL TREVAN: *University of Manitoba, Winnipeg, MB, Canada*
LAURA NELSON: *Alchemy Systems, Austin, TX, USA*
SHELLEY FEIST: *Partnership for Food Safety Education, Arlington, VA, USA*
HELEN TAYLOR: *ZERO2FIVE Food Industry Centre, Cardiff, United Kingdom*
SARA MORTIMORE: *Land O' Lakes, Inc., St. Paul, MN, USA*

Food safety education and training is a large and diverse sphere of activity. Complexity exists in the breadth of students, trainees and stakeholders, as well as in the different types of training and education that are available to the different stakeholder groups. At the same time, measures of training and education effectiveness and impact are many and varied. However, while some people talk about evaluation fatigue and others point to studies showing improvements in knowledge and attitudes following training, there are still limitations to our understanding of both how to attract and best educate the future food safety managers and how to make sure food safety training is effective in instilling the desired food safety skills and behaviours.

Previous symposia have considered capacity building and competency frameworks needed to structure the way forward. This roundtable aims to tackle the next level of detail in terms of practical solutions. Building on the work of the Food Safety Education PDG, this roundtable will engage with food safety professionals and educators to answer key questions about education, training effectiveness and start to resolve food safety education problems. Roundtable panel members will represent groups with interests in educating and training future food safety professionals, current industry personnel and consumers. Speakers will briefly set the scene in their sector areas and audience participation and debate will be encouraged to explore problems and solutions. It is intended that findings of the roundtable session will inform a white paper and action plan to fix current problems with food safety education and enhance food safety training effectiveness.

RT7 I Got an Advanced Degree, Now What?

CHARLES PETTIGREW: *The Procter and Gamble Co., Mason, OH, USA*
CLYDE MANUEL: *North Carolina State University, Raleigh, NC, USA*
HARI DWIVEDI: *bioMérieux, Inc., Hazelwood, MO, USA*
ANDREW CLARKE: *SGS Canada Inc, Etobicoke, ON, Canada*
MOHAMED ZAKI BADAOUI NAJJAR: *PepsiCo, Valhalla, NY, USA*
JOHN LUCHANSKY: *U.S. Department of Agriculture-ARS-ERRC, Wyndmoor, PA, USA*

Graduate students and postdoctoral fellows oftentimes find themselves in a conundrum; on one hand, academia appears to be the next logical step based on their research and education, yet, the academic life does not always feel like a solid fit. Graduate training generally does not provide much exposure to career opportunities outside of academia. Career paths in industry and government remain vague to graduate students due to the fact that these options are not frequently discussed during graduate school and few students obtain industry experience while in graduate school. By allowing new, rising, and seasoned industry and government leaders the opportunity to share their knowledge and experience, this roundtable aims to bridge the gap between academia and the food industry and shed light on how to obtain a career in these sectors. A mixed panel of industry and government leaders will provide the necessary tools and expertise to not only sustain a successful career; but, to also provide young scientists with a fresh perspective and insight on what work life in industry and government entails. Topics for discussion will include, but are not limited to; (a) finding the right company or fit; (b) transitioning from graduate school to industry or government; and (c) expectations of a new hire given one's education and experience post-graduation (skills written on CV that can be transferred into real-life industry skills). The purpose of this roundtable is to make the food industry/government workforce more transparent and to introduce graduate students to employment opportunities in these sectors. To further sustain this communication, the Student PDG is partnering with the Developing Food Safety Professionals PDG to host subsequent webinars geared toward bridging this gap, starting from Fall 2016. These resources move us toward fostering an open line of communication between industry and aspiring scientists.

RT8 Bringing the World Together in the Fight against Listeria monocytogenes: A Regulatory Perspective

JEFFREY FARBER: *University of Guelph, Guelph, ON, Canada*
MICKEY PARISH: *U.S. Food and Drug Administration, Washington DC, D.C., USA*
PETER BEN EMBAREK: *WHO, Geneva, Switzerland*
IAN JENSON: *Meat and Livestock Australia, North Sydney, Australia*
DANIEL ENGELJOHN: *U.S. Department of Agriculture-FSIS, Washington, D.C., USA*

L. monocytogenes continues to pose a major public health risk in developed countries. Its high morbidity and mortality rates make it a prominent food safety concern. Recent outbreaks associated with the consumption of contaminated ice cream, caramel apples, and produce highlight continued challenges in its control, and underscore the need for continued surveillance and regulation. Government efforts to control the presence and persistence of L. monocytogenes in food products and food production facilities differ around the globe, making it difficult to harmonize policies with implication on food trade and commerce. The objective of this session is to present an expert panel view on the current global regulatory frameworks for L. monocytogenes around the world and to discuss potential ways to harmonize and unify such policies.

RT9 Validity of Control Strategies for Hazards in the Supply Chain

ANDREW CLARKE: *Maple Leaf Foods, Etobicoke, ON, Canada*
WENDY WHITE: *Golden State Foods, Conyers, GA, USA*
MICKEY PARISH: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
GILLIAN KELLEHER: *Wegmans Food Markets, Inc., Rochester, NY, USA*
DAVID ACHESON: *The Acheson Group, Salt Lake City, UT, USA*

This roundtable session will convene a panel of professionals from different segments of the supply chain to debate the validity of vendor/supplier controls commonly used for food safety hazards. Certificates of analysis (COAs) can communicate testing results on purchased material, but how reliable are they and are there situations where a COA is enough? The application of testing programs for chemical and/or microbiological hazards within the supply chain has been controversial, so under what circumstances are testing programs appropriate? And who should do the testing? Third-party food safety audits have a long history of use in the food industry to evaluate and qualify suppliers, but what is the role of these types of audits going forward? When is a third party audit enough and when should a company conduct its own audit of its suppliers? Given the supply chain controls required under the Preventive Controls Rules for Human and Animal Food, this session brings a timely debate to these common and sometimes controversial control measures.

Invited panelists will represent ingredient suppliers, manufacturers, retailers, third party auditors, and regulators.

RT10 FDA Food Safety Modernization Act (FSMA) Implementation: What is the Role of Third Party Standards and Audits?

MICHAEL ROBACH: *Cargill, Minneapolis, MN, USA*
JOHN KUKOLY: *BRC, Fenwick, ON, Canada*
RENA PIERAMI: *Mérieux NutriSciences, Chicago, IL, USA*
SHARON MAYL: *U.S. Food and Drug Administration, Silver Spring, MD, USA*

It is increasingly recognized that private third-party audits can play a role in helping the industry to achieve food safety objectives, provided the audits are rigorously performed by qualified, unbiased, auditors who audit to strong food safety standards. These audits are used regularly to meet market requirements imposed by the private sector.

Private third-party audits are often done within the context of private third-party certification schemes. Recognizing the demands on suppliers in terms of compliance with regulatory requirements, as well as conformance to private market requirements, this roundtable will discuss strategic thinking around overlapping public and private requirements, and whether there may be opportunities to minimize redundancies and reduce the burden on business, while upholding food safety outcomes demanded by both the market and FDA.

Thinking on this topic is evolving both in the private sector and at FDA. The food industry seeks clarity of how compliance with third-party standards and audits by third-party auditors can be used by food producers to show their compliance with the FSMA regulations. Owners of certification schemes are considering whether and how to align their standards with FSMA standards. FDA is contemplating whether and how to use reliable, private audits performed outside of FDA's accredited third-party rule. FDA has received numerous comments and questions about this topic at public meetings and

Roundtable

during the FSMA rulemaking process. Given the public interest and upcoming compliance dates for the FSMA rules, this session will provide a timely discussion on a topic of great importance to FSMA implementation and to food safety worldwide.

RT11 How are We Going to Get Everyone Trained for FSMA?

JODI WILLIAMS*: U.S. Department of Agriculture-NIFA Food Safety Division, Washington, D.C., USA*
SAMIR ASSAR*: U.S. Food and Drug Administration - CFSAN, Silver Spring, MD, USA*
DONNA GARREN*: American Frozen Food Institute, McClean, VA, USA*
GERALD WOJTALA*: International Food Protection Training Institute, Battle Creek, MI, USA*

Food safety is a very diverse field and, as such, regulatory changes are critical to the success of public health as a whole. FDA has proposed several major rules to implement the Food Safety Modernization Act (FSMA) that will become final in 2015 and 2016 (http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm247559.htm). Within these rules, emerging opportunities have been identified to incorporate novel preventative training approaches across industry, academia and government. The legislation will impact over 35,000 growers and approximately 189,000 farms in the U.S. and potentially thousands more globally based on the demand of the market. Imported food suppliers will also experience vast changes in training and verification activities. Training in the food industry typically encompass manufacturers, but what about third-parties including community-based organizations (CBOs) and their training needs? Research and teaching are affiliated with academia, but extension programming is also a key component to the delivery of applicable research methods. There are also emerging areas of special interest in the field such as Food Law and Public Policy. The Developing Food Safety Professionals PDG and the Education PDG have come together to build upon the demand for food safety professional training that meets the federal guidelines of the new Food Safety Modernization Act (FSMA). Panelists in this session will include representatives from each of these areas to address the methods in which FSMA training will be implemented across the board. This roundtable will provide a broader perspective of new opportunities for collaboration, training, and outreach established by the government (FDA and USDA-NIFA), industry, and academia. A diverse panel will share their thoughts on innovative training methods designed to reach small processors, beginning farmers and ranchers and the coordinated strategies that are underway towards the implementation of FSMA. New approaches to training that are on the horizon for the industry will also be shared.

RT12 Intervention, Development, and Evaluation of Mixed-method Approaches for Retail, Consumer and Food Service

ANGELA FRASER*: Clemson University, Clemson, SC, USA*
CATHERINE CUTTER*: The Pennsylvania State University, University Park, PA, USA*
YAOHUA FENG*: University of California - Davis, Davis, CA, USA*

As food safety communication and education intervention evaluation expands to increased behavior-focused outcomes, there are opportunities to improve the design and reporting of research. A recent review of food safety interventions designed for food service audiences demonstrated that there was a wide variety in the foundation for interventions, evaluation research design, and methodology selection - ultimately resulting in a wide variety of quality outputs. The goal of this session is to facilitate a discussion among researchers who design and evaluate interventions and are familiar with mixed methods approaches including focus groups, long interviews, observation, ethnography, mental models and content analysis. Through the roundtable format a facilitated discussion of how to design higher quality studies and create a set of standards for these approaches to move the domain forward. This session can help set the stage for researchers to create higher quality interventions and evaluations to improve the outputs of the domain and ultimately impact public health.

RT13 Campylobacter: Can We Solve the Problem?

MARTA CERDA-CUELLAR*: IRTA-CReSA, Barcelona, Spain*
CATHERINE CARRILLO*: Canadian Food Inspection Agency, Ottawa, ON, Canada*
MAARTEN NAUTA*: DTU, Copenhagen, Denmark*

There have been major advances in the understanding of the epidemiology of Campylobacter. However, control of Campylobacter has so far not been very successful and we still have limitations in quantifying the contribution of each known food reservoir on the public health impact of this foodborne disease. Some of the advances and also limitations include quantitative risk assessment and risk-based microbiological criteria and sampling tools; the methods that we currently use to detect and enumerate this organism in foods; the public health report system; the tracking of this pathogen through the food chain, and the limited interventions available to control this pathogen in food animals. This roundtable has been organized by member of several PDGs and will comprise a group of experts from the industry, academia and regulatory agencies. These experts are working in different aspects of this complex issue but with the same goal of reducing the public health impact of Campylobacter. Participants will have the opportunity to learn the latest information about Campylobacter through a panel discussion with key questions, some of which will be:

- Why is campylobacteriosis still so prevalent?
- Are we tackling the right sources of transmission?
- What are the most appropriate sampling methods to detect Campylobacter in foods?
- Are the intervention methods really reducing this pathogen in foods?
- What do we consider the most promising interventions?
- What can we learn from the experiences in other countries?

Symposium Series on Food Microbiology

Sponsored by the
ILSI North America
Technical Committee on Food Microbiology

in conjunction with the
International Association for Food Protection

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.

RT5 A Debate: Current Perspectives in Food Safety

This session is dedicated to the memory of John Cerveny.

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: "Is our food supply too clean? Are there unintended health consequences as we chase zero?"; "Does the food industry need to change its approach to food safety management, taking into account cultural differences between boomers and millennials?"; and, "Is there a human health risk introduced from the use of chemical sanitizing agents in food manufacturing that exceeds the benefit from the reduction of microbial risk that is achieved?" Each topic will include a 7-minute presentation in support of (YES) followed by a 7-minute presentation in opposition of (NO) 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.

Is Our Food Supply Too Clean? Are There Unintended Health Consequences as We Chase Zero?

JEFF LEJEUNE, *Ohio State University*

Abstract not provided at time of print.

JOE STOUT, *Commercial Food Sanitation, LLC*

Abstract not provided at time of print.

Does the Food Industry Need to Change Its Approach to Food Safety Management, Taking into Account Cultural Differences between Boomers and Millennials?

BENJAMIN CHAPMAN, *North Carolina State University*

As workforces change and technology becomes an increasing part of the daily communication system, food safety professionals may struggle with connecting with and leading millennials. An organization is only as good as its weakest link and the world of food safety hinges on employees' performance. In this debate, Chapman will set the stage for challenges and opportunities to shift approaches in communicating with newer generations. The discussion will focus on communication style, work-life balance, value systems, transparency and feedback.

KELLY STEVENS, *General Mills Inc.*

Abstract not provided at time of print.

Is There a Human Health Risk Introduced from the Use of Chemical Sanitizing Agents in Food Manufacturing That Exceeds the Benefit from the Reduction of Microbial Risk That is Achieved?

MICHAEL HOLSAPPLE, *Michigan State University*

Chemicals are routinely used to sanitize and disinfect food contact surfaces so that they are as free as possible from microorganisms that can cause foodborne illness. There is a difference between "disinfecting", which means to destroy or irreversibly inactivate specified infectious microbes, and "sanitizing", which means to reduce; but not necessarily eliminate microorganisms from surfaces. In most cases, these products are registered for use as pesticides with the US EPA. Once applied, the allowable residues and the monitoring thereof in food processing and preparation are the responsibility of the US FDA. The tasks of ensuring that the chemicals are prepared and applied properly to avoid toxicity and inappropriate residues rests with the food processor and foodservice operator. Chemicals in some sanitizers can be irritating to the skin or can cause rashes, or are corrosive and can cause severe burns if splashed on the skin or in the eyes. These human health risks underscore the importance of MSDSs, and of worker training. The efficacy of a chemical used for sanitizing rests upon its ability to reduce the contamination level. The standard for contamination reduction of food contact surfaces is generally accepted as 99.999% (a 5-log reduction) achieved in 30 seconds. A 5-log reduction still means that of 1,000,000 microbes present, 10 have survived. Any time a chemical is used to produce microbial mortality, the possibility of promoting resistance exists. Any action that contributes to potential antibiotic resistance is not only a human health issue, it is a matter of national security.

KATHERINE MJ SWANSON, *KMJ Swanson Food Safety, Inc.*

The human health risk introduced from the use of chemical sanitizing agents in food manufacturing does not exceed the benefit from the reduction of microbial risk that is achieved. This presentation will briefly address regulatory requirements for use of chemical sanitizing agents in food. The benefits of chemical sanitizing agents in both food contact and non-food contact applications will be illustrated using examples where lack of sanitizing chemicals contributed to significant foodborne illness outbreaks. The conclusion that the human health risk introduced from the legal use of chemical sanitizing agents in food manufacturing does not exceed the benefit from the reduction of microbial risk that is achieved is supported by the following evidence: 1) regulatory review of chemical sanitizing agents used in food manufacturing include risk evaluation prior to approval, 2) lapses in basic sanitation practices have long been associated with foodborne illness outbreaks, and 3) application of chemical sanitizing agents can help reduce the spread of microbial contaminants to uncontaminated surfaces, including food, thus improving the overall safety of the food supply.

Pesticide use include rigorous evaluations of chemical safety prior to product registration

The Environmental Protection Agency (EPA) regulates all antimicrobial pesticides, which include chemical sanitizing agents. If EPA determines that use of the product would result in residues of the chemical in food, the product cannot be registered unless they determine that there is "a reasonable certainty of no harm" from the exposure to the residue in food and from other non-occupational sources. When EPA determines that a pesticide product can be registered for use, the Agency has concluded that the use of the pesticide product will not cause unreasonable adverse effects to humans or the environment when applied according to the label directions and restrictions. In addition to EPA product registration requirements, many states have regulation and registration requirements that must be met. These vary from state to state, with some requirements being more stringent than EPA requirements.

S21 Cyanotoxins in the Water Supply and Potential Food Safety Ripple Effects

Microcystin is a type of cyanotoxin and is one of the several toxins produced by the cyanobacteria otherwise known as blue-green algae. The presence of high concentrations of microcystin in potable water recently became a serious health risk in the United States with potential impact on the food industry. Increasing levels of cyanotoxins in water has also been recognized as an emerging global issue with potential implications for irrigation water used in agriculture. The combination of increased surface water temperatures and increased nutrient levels from farm and waste water run-off are expected to be the primary drivers for increased algae blooms. Both the U.S. EPA and the World Health Organization have devised advisory or provisional guidance values for microcystin in potable water supplies to support water monitoring. As algae blooms continue to grow in severity presenting an environmental challenge not just in the United States but globally, there is a need for increased research and awareness on cyanotoxins as well as effective mitigation programs and development of analytical methods to adequately identify each toxin. This information will be beneficial in supporting global public water system managers who are on the front lines of this issue, ensuring that our potable water supply continues to be safe for human consumption and use in food manufacture. In this symposium, we will review cyanotoxins and their effects on human health, potential impacts to the food industry including potable water used in food production and irrigation for agriculture, discuss monitoring activities, occurrence rates, action levels set by global regulatory agencies, and explore applicable mitigation methods.

Cyanotoxins: An Emerging Global Issue

KELLY MAGURANY*, ConAgra Foods, Naperville, IL*

Cyanobacterial blooms are becoming an increasingly prevalent occurrence around the world with major incidents occurring recently in the United States. As a means to mitigate impact to the potable water supply, the US Environmental Protection Agency has issued health advisory levels for the major cyanotoxins of concern, as well as providing guidance on mitigation techniques to source water managers. As awareness to potable water quality increases, impact on the food industry may become an emerging and important topic for consideration.

Regulatory Perspective and Associated Human Health Effects with Cyanotoxin Exposure

LESLEY D'ANGLADA*, U.S. EPA, Washington, DC*

Harmful Algal Blooms, especially cyanobacterial blooms, are of concern for freshwater systems because of their potential to produce toxins, also known as cyanotoxins, and the possible adverse impacts on drinking and recreational waters. In order to protect human health from exposure to cyanotoxins in drinking water, EPA published Drinking Water Health Advisories for the cyanotoxins microcystins and cylindrospermopsin, and Health Effects Support Documents for microcystins, cylindrospermopsin and anatoxin-a. Health advisories describe non-regulatory concentrations of cyanotoxins at or below which adverse health effects are not anticipated to occur over a period of 10-day exposure. EPA recommends health advisory levels at or below 0.3 micrograms per liter for microcystins and 0.7 micrograms per liter for cylindrospermopsin in drinking water for children pre-school age and younger (less than six years old). For school-age children through adults, the recommended HA levels for drinking water are at or below 1.6 micrograms per liter for microcystins and 3.0 micrograms per liter for cylindrospermopsin. Health Advisories are developed for young children since children younger than 1-year-old are more susceptible than older children and adults as they consume more water relative to their body weight. Exposure to the HABs and toxins via drinking water ingestion can result in adverse health effects including liver damage from exposure to microcystin, and kidney damage from exposure to cylindrospermopsin in drinking water. Other health effects such as reproductive damage, neurological damage, and gastrointestinal illness were also associated with exposure to cyanotoxins in drinking water. More research is needed to assess the carcinogenic potential of these cyanotoxins.

Mitigation of Cyanotoxins (Microcystin)

RICHARD LORENZ*, Ohio State University, Westerville, OH*

Harmful Algal Blooms, especially cyanobacterial blooms, are of concern for freshwater systems because of their potential to produce toxins, also known as cyanotoxins, and the possible adverse impacts on drinking and recreational waters. In order to protect human health from exposure to cyanotoxins in drinking water, EPA published Drinking Water Health Advisories for the cyanotoxins microcystins and cylindrospermopsin, and Health Effects Support Documents for microcystins, cylindrospermopsin and anatoxin-a. Health advisories describe non-regulatory concentrations of cyanotoxins at or below which adverse health effects are not anticipated to occur over a period of 10-day exposure. EPA recommends health advisory levels at or below 0.3 micrograms per liter for microcystins and 0.7 micrograms per liter for cylindrospermopsin in drinking water for children pre-school age and younger (less than six years old). For school-age children through adults, the recommended HA levels for drinking water are at or below 1.6 micrograms per liter for microcystins and 3.0 micrograms per liter for cylindrospermopsin. Health Advisories are developed for young children since children younger than 1-year-old are more susceptible than older children and adults as they consume more water relative to their body weight. Exposure to the HABs and toxins via drinking water ingestion can result in adverse health effects including liver damage from exposure to microcystin, and kidney damage from exposure to cylindrospermopsin in drinking water. Other health effects such as reproductive damage, neurological damage, and gastrointestinal illness were also associated with exposure to cyanotoxins in drinking water. More research is needed to assess the carcinogenic potential of these cyanotoxins.

S31 The Rise of the Genomes – Improving Health through Better Food Quality and Food Safety

This session follows up on the past ILSI North America sponsored, "Rise of the Genomes," sessions in 2014 and 2015; with a focus on the applications of next generation sequencing methods, including whole genome sequencing for microbial source tracking, RNA-seq and metagenomics. Whole genome sequencing is becoming a routine tool to track foodborne pathogens from the farm to the table to identify and control outbreaks quickly and efficiently. The tool is also increasingly used by the industry to track pathogens in the production environment and characterize beneficial bacteria used in the food production, e.g., starter cultures and probiotics. However, bacteria in the gut are not just associated with foodborne infections. The composition of the gut flora, the gut microbiome, is also related to illness, e.g., cancer and atherosclerosis and the composition of the microbiome is dependent on and may be altered through dietary changes. Next generation sequencing is also used to study the microbiome through metagenomics and RNA-seq can be applied to study how a microorganism thrives in a given niche.

This symposium will include an overview of the application of next generation sequencing, Two talks by representatives of the food industry on their experiences using whole genome sequencing to improve food and food safety, a talk on the use of RNA-seq to elucidate genetic elements and pathways used by pathogens to thrive in food environments, and talks about the use of metagenomics to study the impact of the microbiome on health.

Overview of Next Generation Sequencing

PETER GERNER-SMIDT, *Centers for Disease Control and Prevention, Atlanta, GA*

During the last decade, the sequencing technology has evolved making it possible to sequence the full genome of a microorganism in a matter of hours at a cost that equals that of many other molecular methods used in the laboratory. At the same time the IT-infrastructure and bioinformatics disciplines have evolved making it possible to store, analyze, share and communicate the huge amounts of data generated. Next generation sequencing is based on the principle of massive parallel sequencing where the genome is sequenced multiple times in random small pieces which subsequently are assembled and analyzed using bioinformatics software. The technology is increasingly used in academia and is transforming microbiology in many public health and food regulatory laboratories by replacing many of the traditional phenotypic and molecular methods currently in use. A number of characteristics of a microorganism can be directly be predicted from its sequence, e.g., its genus and species, serotype, virulence profile and resistance to antimicrobials and disinfectants. Whole genome sequencing has also proven to have superior discrimination and precision compared to other subtyping methods, e.g., PFGE. This makes the method almost ideal for source tracking and to study transmission chains in a food production facility. For this reason it is also being adopted by food industry laboratories. Currently, the technology is mainly used to characterize microorganisms in pure culture but it is increasingly also being used to characterize complex populations of microorganisms in clinical and food samples (metagenomics).

Implications of Whole Genome Sequencing Findings to the Food Industry

DEANN AKINS-LEWENTHAL, *ConAgra Foods, Omaha, NE*

Recent advances in next generation sequencing and bioinformatics tools have made whole genome sequencing a viable tool for foodborne bacterial pathogen surveillance. Whole genome sequencing is being investigated by the food industry as a new tool that can be used for source tracking potential pathogens in the manufacturing plant environment. This presentation will discuss possible applications of whole genome sequencing in the food industry as well as review the regulatory impact of whole genome sequencing on the industry. Two case studies from manufacturing facilities will be given to illustrate how whole genome sequencing played a role in pinpointing the source of potential pathogens in the food environment. These case studies will highlight how challenges in manufacturing environments have led to new opportunities to use whole genome sequencing as an investigational tool. Lastly, this session will discuss potential implications of whole genome sequencing on the food industry that risk managers should consider when using this technology.

Microbial Source Tracking

ROBERT C. BAKER, *MARS Incorporated, McLean, VA*

Abstract not provided at time of print.

RNA-seq of Pathogen Transcriptomes in Food and Food Associated Environments

MARTIN WIEDMANN, *Cornell University, Ithaca, NY*

Next generation sequencing (NGS) tools are increasingly being recognized for their ability to provide for improved subtyping of foodborne pathogen isolates as well as for metagenomics-based characterization of all DNA present in a given sample; the later approach in particular can provide both intriguing and sometimes potentially misleading information. NGS tools also provide a powerful approach to characterizing all RNA present in a given microbial population exposed to either food relevant environmental conditions or present in an actual food matrix. In these applications, termed "RNA sequencing (RNA-seq)", NGS provides significant advantages over microarray technologies, which until recently have been predominantly used for characterization of transcriptomes. This presentation will provide examples of how RNA-seq has provided new insights into foodborne pathogen responses to food associated stress conditions and how it has been used to elucidate the physiological state of pathogens in foods. This presentation will also highlight how this information can be translated into improved control strategies using a "precision food safety" type strategy that can custom tailor interventions to different food types and commodities. Ultimately, these approaches may allow us to move to food safety approaches that not just attempt to reduce pathogen numbers in foods, but also will assure a pathogen physiological state that is associated with a reduced public health risk (such as a state that reduces or eliminates production of relevant toxins). In addition to characterization of pathogen transcriptomes, there also is considerable interest in characterization of food meta-transcriptomes, including to address concerns about metagenomics-based detection of genetic material from non-viable organisms. While meta-transcriptomics approaches, alone as well as in combination with metagenomics, have considerable potential for improved characterization of foods and raw materials, including detection of potential food safety and adulteration incidents, considerable additional data and research is needed to develop these tools and to allow for reliable interpretation of results.

The Human Microbiome in Health and Disease

VINCENT B. YOUNG, *University of Michigan, Ann Arbor, MI*

The application of approaches and techniques initially explored to study complex microbial communities in environments such as soil and seawater has revolutionized our thinking about our indigenous microbial communities. We are beginning to develop a greater understanding of how the microbiome can play an essential role in human health and disease. In this talk, I will briefly introduce how the development of next-generation sequencing platforms has facilitated the culture-independent study of the indigenous microbiota with a focus on studies of the gastrointestinal tract. Work that has associated the gut microbiota with various diseases states will be reviewed. This will include a discussion of association studies in patients as well as the use of animal models for mechanistic studies of how the microbiome can influence host physiology. In addition to discussing how sequence-based approaches can facilitate microbiome research, I will also include a discussion of the continued role that microbial cultivation still plays in a comprehensive study of the role of the microbiome in maintaining human health and triggering disease states.

The Impact of Diet on the Human Microbiome

GARY WU, *University of Pennsylvania, Philadelphia, PA*

Abstract not provided at time of print.

S43 How Do I Validate That? Assuring Credibility of Non-thermal and Novel Thermal Controls for Microbiological Hazards

Validation of hazard control measures is an important requirement of food safety management systems, and FSMA is amplifying the urgency of such validations. However, the vast majority of prior work and information available has focused primarily on reduction and control of microbial pathogens via thermal processes. In contrast, criteria for the design, execution, and interpretation of validation studies for non-traditional/non-thermal controls often are unclear or simply unavailable. A wide-range of products are subjected to process steps that provide pathogen reduction by solely non-thermal means, such as high-pressure processing, varied wavelength light exposure, and/or radio frequency treatment. This session discusses approaches to validation of a selection of non-thermal control measures.

Essential Criteria for Making a Non-thermal Validation Study Acceptable to a Regulator

NATHAN ANDERSON, *U.S. Food and Drug Administration-IFSH, Bedford Park, IL*

Emerging technologies hold great potential for reducing the microbiological risk while meeting increased consumer demands for fresh, minimally-processed, clean-label foods. This presentation will discuss considerations for validating process efficacy of non-thermal technologies. Participants should be able to describe regulatory expectations for process validations, identify key variables to monitor, control and record, and understand the key elements of a thorough validation report.

Validation of Ingredient-based Systems to Control Pathogens

KATHLEEN GLASS, *University of Wisconsin-Madison, Madison, WI*

The safety of a processed low-acid food is assured by controlling acidity, water activity, antimicrobials, and processing or storage temperatures. Additive or synergistic interaction of these factors can lead to lower usage levels of added antimicrobial agents, and thereby enhance the sensory and functional properties of the food. Due to the complex interaction among these factors, predictive models generated in laboratory media may be insufficient to justify choices of control measures when creating a food safety plan. Therefore, identifying relevant parameters and limits require microbial challenge studies in specific foods. Groups such as the National Advisory Committee for Microbiological Criteria of Foods (NACMCF), Health Canada, and New South Wales Food Authority provide guidelines for conducting challenge studies. However, even experts food microbiologist may overlook the significance of intrinsic antimicrobial factors or the impact of microbial ecology of a food that change as formulations and ingredients evolve. This presentation will highlight the basics of conducting challenge studies and provide examples of factors that may be unrecognized as being critical for safety.

Radio Frequency: New Technology Applications and Validation of Pathogen Reduction

JEYAMKONDAN SUBBIAH, *University of Nebraska-Lincoln, Lincoln, NE*

Radiofrequency (RF) processing is a novel thermal processing technology. It is a form of dielectric heating in which the applied electromagnetic field vibrates polar molecules (usually water) and ions within the food material causing volumetric heating. RF processing can be effectively used to reduce the come-up time (the time for the coldest spot in the food to reach the desired temperature) due to its larger penetration depth. Validation of this novel thermal processing method has some unique challenges such as identification of cold spots and temperature measurements, when compared to traditional thermal processing methods. Validation of RF processing for pathogen reduction requires following steps. First, thermal inactivation kinetics of pathogens in food product are first determined. Using the dielectric and thermo-physical properties of food products, multiphysics models can be used to optimize electrode and package shape for uniform heating. Multiphysics models are then used to determine the cold spot locations. Fiberoptic sensors are used to verify cold spot locations using experiments. Food quality is then evaluated at multiple time-temperature combinations that achieved desired pasteurization levels. Finally, microbial challenge studies are performed at the final time-temperature combination that provided the highest food quality. Pasteurization of egg white powder and wheat flour using RF processing will be presented as case studies.

Cold Plasma: A Case Study in Critical Factors Affecting Development and Validation of a Novel Technology

BRENDAN NIEMIRA, *U.S. Department of Agriculture-ARS, Wyndmoor, PA*

Contamination of fruits, vegetables, meats, poultry, seafood and other products by foodborne pathogens has prompted research into novel interventions. Cold plasma is a nonthermal food processing technology which uses energetic, reactive gases to inactivate contaminating microbes. This flexible sanitizing method uses electricity and a carrier gas such as air, oxygen, nitrogen or helium; antimicrobial chemical agents are not required. In order to validate cold plasma as a viable intervention for foods and food contact surfaces, and to support development of the technology from the laboratory to the pilot scale and ultimately to commercialization, key elements related to the performance, efficacy, and regulatory standing must be identified and addressed. As an emerging technology, a variety of competing cold plasma equipment designs are under development in research environments worldwide. Unlike other relatively new antimicrobial technologies such as high pressure processing, pulsed light or precision UV, which offer different paths to the same intervention endpoint, the nature of plasma is highly dependent on the equipment used to produce it. This, in turn, has critical impacts on the predominant modes of action for inactivating pathogens, contaminants and spoilage organisms. This presentation will summarize the science behind cold plasma, describe the major classes of cold plasma generation equipment, their impact on efficacy and modes of action, and list critical factors related to development and validation of cold plasma.

Technical Abstracts

T1-01 Laboratory Accreditation – Progress Towards the Nation's Integrated Food/Feed Safety System

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Introduction: Laboratory accreditation attests to the competency and technical capability of a laboratory, leading to results which are defensible to a recognized standard, and supports the traceability and accountability of results generated by a laboratory that may be made available for consideration by federal agencies for enforcement actions. ISO/IEC 17025 is an accreditation standard utilized by laboratories throughout the world.

Purpose: The Food Safety Modernization Act of 2011 stresses the importance of quality testing standards and directs FDA to establish a program for laboratory accreditation. This work furthers that effort through collaboration with three national associations and their member governmental laboratories.

Methods: In 2012, FDA entered into five-year cooperative agreements with 31 state food-testing laboratories to either attain ISO/IEC 17025:2005 accreditation (23 laboratories/$300,000/year) or expand and maintain existing accreditation (8 laboratories/$150,000/year). At the same time, FDA awarded a five-year cooperative agreement to APHL to support accreditation in collaboration with AFDO and AAFCO. In 2015, an additional cohort of 6 food and 20 feed testing laboratories was awarded funding to obtain accreditation.

Results: The first cohort of laboratories (N=23) will be accredited by the end of 2017. Among other support, APHL, AFDO and AAFCO have provided these laboratories with a Discussion Board, >200 documents posted to a resource website, >15 webinars posted providing training to >800 participants, targeted assistance to 16 unfunded laboratories, development of a Laboratory Curriculum Framework for Governmental Food and Feed Testing Laboratories, and publication of GOODSamples (Guidance for Obtaining Defensible Samples).

Significance: Investment in governmental laboratory accreditation for the nation's regulatory food and feed testing laboratories will provide added value towards the mission of protecting the public health. Accreditation leads to greater laboratory capacity and improved quality of data submitted to regulatory food agencies. With all of the efforts above, the number of accredited laboratories performing regulatory testing will be significantly increased.

T1-02 Microbial Inoculation of Powdered Infant Formula for Quality Assurance Studies

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❖ ***Developing Scientist Competitor***

Introduction: Focus on quality assurance within microbiological laboratories has increased due to new federal regulations and foodborne disease outbreaks. Check samples and reference materials can be used to ensure testing capabilities of microbiological laboratories. Availability of standards to use for check samples is limited due to requirements regarding long term stability, homogenous distribution of microorganisms, and storage conditions.

Purpose: A method that achieves homogenous and stable inoculation of powdered infant formula (PIF) samples at realistic levels for use in quality assurance (QA) studies is summarized. This method was used to inoculate PIF with pathogens of concern including *Salmonella* and *Cronobacter*.

Methods: Dry inocula were prepared by spraying liquid inocula of target organisms over PIF using an ultrasonic atomizing spray nozzle while mixing. Serial dilutions of dry inocula with uninoculated PIF were then made to prepare final samples. Homogeneity and stability of target organisms in dry inocula as well as final samples were confirmed by quantitative testing and statistical analysis.

Results: Within test portions, the mean analytical standard deviation for PIF inocula was 0.37 log CFU/g and 0.25 log CFU/g for final samples. Mean heterogeneity standard deviation between test portions for PIF inocula was 0.02 log CFU/g and 0.05 log CFU/g for final inoculated samples. Heterogeneity standard deviation in both inocula and final samples were not significantly different than zero according to F-tests, indicating sufficient homogeneity. Total aerobic counts in *Cronobacter* inocula PIF decreased by 0.25 log CFU/g over one month and fulfilled the expanded criteria for stability according to ISO 13528.

Significance: Statistical analysis of homogeneity and stability data supports that the inoculation method used in these studies is a reliable technique to artificially contaminate PIF. This method may be used to prepare reference materials and check samples for QA studies in microbiological laboratories.

T1-03 Reducing Enrichment Time and Selective Media to Isolate Environmental *Listeria monocytogenes* or *L.* spp. Decreases Costs and/or Time to Results

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Introduction: Classic detection methods are time, labor, and resource intensive. Environmental testing for *Listeria monocytogenes* and *L.* spp. isolates requires selective enrichment and multiple selective-differential media; presumptive positives must be verified by PCR.

Purpose: This study evaluated the equivalence of reducing media and/or enrichment time to a widely used modified FDA Bacteriological Analysis Manual (BAM) method.

Methods: Environmental samples (*n*=2634) were collected from food, non-food, and transfer point contact surfaces from retail delis. Each sample was tested for *L. monocytogenes* and *L.* spp. using a modified BAM protocol. The results were recorded for two enrichment periods (24 h, 48 h) plated concurrently on modified Oxford agar (MOX) and *Listeria monocytogenes* Plating Medium (LMPM) incubated 48 h. Outcomes from reduced enrichment time and single media type were compared to responses from the complete modified BAM method; 90% confidence intervals (α=0.10) were constructed using two-tailed asymptotic tests to evaluate equivalence at ±1.0%, ±0.5%, and ±0.1% prevalence.

Results: To detect *L.* spp., equivalence at ±1% prevalence was achieved plating 24 h enrichments on both media and 24 h and 48 h enrichments plated on MOX. No abbreviated methods were equivalent at ± 0.5% prevalence for *L.* spp. To detect *L. monocytogenes*, plating 24 h and 48 h enrichments to LMPM was equivalent at ±0.5% (α=0.10). At ±0.1% prevalence, truncated methods lost equivalence due to increases in false positives. MOX did not significantly contribute to detection of *L. monocytogenes* when LMPM was used.

Significance: Truncated methods to isolate *L. monocytogenes* or *L.* spp. reduce costs and/or time to results and may be an appropriate option for environmental monitoring plans if increased false positives are more tolerable.

T1-04 Rapid Quantitative Detection and Genotyping of *Staphylococcus aureus* in Retailed Frozen Flour and Rice Products

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Introduction: Frozen flour and rice products are easily been contaminated by *Staphylococcus aureus*. The contamination of *S. aureus* to frozen flour and rice products is allowed in a limited amount such as below 10^4 CFU/g, however, the corresponding quantitative method for *S. aureus* is plating-count based, which is time- and labor-consuming. What's more, there is a data gap on the contamination of S. aureus to frozen flour and rice products.

Purpose: It is aimed to establish a nanomagnetic-PCR based quantitative method for *S. aureus* without pre-enrichment and investigate the contamination level of *S. aureus* in retailed frozen flour and rice products.

Methods: A new rapid quantitative detection method for the *S. aureus* contamination in food samples was built up by combining nano-magnetic separation with *Taq*Man Real-time PCR. Frozen flour and rice products samples (meat-stuffing, vegetable-stuffing and non-stuffing) were collected to isolate and identify the presence of *S. aureus*. All the *S. aureus* isolates were screened for their enterotoxin gene diversity by PCR amplification of 18 enterotoxin genes (*sea-see*, *seg-ser* and *seu*), and Multilocus Sequence Typing (MLST) and eBURST analysis.

Results: In total, 288 frozen samples were collected and the total positive rate of *S. aureus* was 30.56% (88/288). After confirmation, 124 isolates were acquired. The new rapid nanomagnetic-PCR based quantitative method was compared with the reference plating method in 32 *S. aureus* positive samples, and there was no remarkable difference ($p>0.05$) between the two methods. The meat-stuffing samples had the highest contamination rate of 41.44% and the highest contamination level which ranged from 2.00×10^3 to 1.21×10^5 CFU/g. Six out of 88 positive samples exceeded the allowable limit in the new national standard (10^4 CFU/g).

Among the 124 isolates, 89.52% (111/124) were positive for enterotoxin genes. Among these positive isolates, 5 maximally contained 10 enterotoxin genes. The *sep* gene was the most detected (44.35%, 55/124), followed by *sei* (43.55%, 54/124) and *seg* (42.74%, 53/124). The isolates were scattered in 36 STs including 5 new ST types and attributed to 19 CCs. ST-7 and CC7 represented the largest ST (27/124) and clonal complex (37/124). And ST-7 had the most diversified enterotoxin genes. The five traditional enterotoxin genes (*sea-see*) were almost distributed in 4 CCs (CC1, CC5, CC25 and CC7).

Significance: It can be used for food safety monitoring and risk assessment.

T1-05 Comparison of Rapid Detection Methods of *Salmonella* Enteritidis and *E. coli* O157:H7 in Cookie Dough

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❖ ***Developing Scientist Competitor***

Introduction: Cookie dough is recognized as a potential vehicle for *Salmonella* for using egg as one of its ingredients. In 2009, commercial raw cookie dough was also reported as a novel vehicle for *E. coli* O157:H7 transition. Rapid detection methods for pathogens in cookie dough are in critical need to prevent foodborne outbreak and ensure food safety.

Purpose: The goal of this study is to compare rapid detection essays, including multiplex PCR and real-time PCR, to standard culture method for their sensitivity in detecting *Salmonella* and *E. coli* O157:H7 in cookie dough.

Methods: Samples of artificially inoculated cookie dough were incubated at 37°C for up to 24 hours. In culture method, *Salmonella* and *E. coli* O157:H7 were detected by selective plating on selective media. In multiplex PCR, *invA* and *hilA* genes were used to detect *Salmonella*; *stx*1, *stx*2, and *rfbE* gene were used to detect *E. coli* O157:H7. For real-time PCR, SureTect Pathogen Detection kit was used with PikoReal real-time PCR system for both pathogens. While various concentrations of pathogens in cookie dough were tested, we also compared the total assay time required to detect 1 CFU/20 g for each assay.

Results: Culture methods were able to detect 10^4 CFU/20g *Salmonella* and 10^3 CFU/20g *E. coli* O157:H7 in 24 hours. Multiplex and real-time PCR identified both pathogens at 10^5 CFU/20g after 8 hours and 1 hour, respectively. In order to detect 1 CFU/20g of pathogens in cookie dough, culture method required 33 hours and 36 hours for *Salmonella* and *E. coli* O157: H7, respectively. Multiplex PCR were able to detect 1 CFU/20 g of pathogens in 14 to 20 hours including enrichment. For real-time PCR, 1 CFU/20g of both pathogens were detected in 14 hours.

Significance: This study demonstrated that culture method and both PCR methods were reliable to detect *Salmonella* and *E. coli* O157:H7 in raw cookie dough. However, PCR-based methods were able to detect pathogens in cookie dough more rapidly than culture-based method. PCR-based methods would be a alternative to standard culture-based method for pathogen identification.

T1-06 Detection of Shiga Toxin-producing *Escherichia coli* by Linkage Analysis of Genomic Co-linear Markers Utilizing Droplet Digital PCR

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Introduction: Pathogenic STEC are defined by the concomitance presence of virulence genes such as *stx* and *eae*. However, when these markers are detected by PCR, the testing should continue until it is definitely proven that they originated from the same bacteria and not from two different bacteria in the sample bearing one of each. Confirmatory methods are cumbersome as the prevalence of these markers leads to a high rate of presumptive positives.

Purpose: The purpose of this study was to evaluate droplet digital PCR as a method to unambiguously identify STEC by detecting and co-localizing genomic markers.

Methods: A culture of E. *coli* characterized by the presence of the two genomic markers, *O26* and *eae,* was enumerated on TCS plates and partitioned into nanoliter volume droplets. Droplets were submitted to various treatments including heating, enzymatic treatment, and exposure to antibacterial peptides, intended to lyse encapsulated bacteria. Duplex PCR amplification of both *O26* and *eae* markers was performed and results were analyzed according to a linkage detection method based on the observation that presence of co-linear markers will increase the number of double-positive droplets relative to the number expected due to chance. A control experiment was run with a mixture of strains bearing one marker.

Results: Bacteria enumeration obtained by PCR positive droplet counting yielded a result matching the initial plate enumeration. Moreover, while no linkage was observed between genes from independent strains, mathematical analysis indicated a significant linkage for double positive bacteria. Optimization of the in-droplet lysis allowed to recover >90% of linkage, thereby demonstrating that ddPCR was able to adequately detect bacteria displaying co-linear makers.

Significance: These results suggest that ddPCR may be a straightforward method to detect in a single PCR run the presence of bacteria such as pathogenic STEC characterized by the concomitant presence of several markers.

T1-07 A PCR-based, Rapid Screening Assay for the Detection of Temperate Phage Integrases and Evaluation of Genome Diversity in *Salmonellae*

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❖ ***Developing Scientist Competitor***

Introduction: Temperate phages constitute a source of genetic diversity by encoding virulence factors such as toxins, effector proteins, and adhesion factors as well as antibiotic resistance genes that alter the bacterial fitness of their host. Within the *Enterobacteriaceae* family, the integration of prophages into their bacterial hosts is mediated by site-specific tyrosine integrases.

Purpose: The objective of this study was to assess if a PCR assay designed to detect tyrosine phage integrases of the *Enterobacteriaceae* family could be used as a rapid screening tool to assess genome diversity in *Salmonellae*.

Methods: A PCR assay designed against thirty-two enteric phage tyrosine integrase sequences located in GenBank was used to assess the presence of phages within thirty rare serotypes of *Salmonella* (one isolate per serotype) of foodborne origin and ten clinical isolates of *Salmonella* Enteritidis. The whole genomes of the *Salmonella* isolates were sequenced and the bioinformatics tools PHAST and PhiSpy were used to detect phages within the genomes.

Results: Within the 40 *Salmonella* isolates, the PCR assay detected 120 phage integrases compared to the bioinformatics programs PHAST and PhiSpy, which detected 161 and 183 phages, respectively. The PCR assay detected more numerous and diverse phage integrases within the rare *Salmonella* isolates than within *Salmonella* Enteritidis isolates. This was validated by PHAST, which identified 48 different phages, within the forty isolates, originating from *Escherichia coli, Salmonella* spp., *Shigella* spp., *Klebsiella* spp., *Burkholderia* spp., and phage elements with homology to *Vibrio spp.* Only four of these phages were present in each of the ten *Salmonella* Enteritidis isolates.

Significance: This study demonstrates the potential use of this PCR assay as a rapid screening tool to assess the diversity of temperate phages in *Salmonella* spp. isolated from food sources. This study also highlights *Salmonella* Enteritidis as a highly conserved serotype with little genetic diversity.

T1-08 Evaluation of Real-time PCR Combined with Immunomagnetic Separation or Centrifugation for Detection of Low Levels of Healthy and Sanitizer-Injured *Salmonella* spp. on Mung Bean Sprouts

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Introduction: Mung bean sprouts, which are used extensively in Asian cuisine, are now well recognized to harbor of *Salmonella* spp., and are implicated in salmonellosis outbreaks. To ensure the safety of bean sprouts consumption, thus the presence of *Salmonella* spp. needs to be screened using an accurate and rapid method.

Purpose: The aim of this study was to develop a rapid and accurate detection methodology for low levels of healthy and sanitizer-injured *Salmonella* on mung bean sprouts using real-time PCR coupled with either immunomagnetic separation (PCR-IMS) or centrifugation (PCR-cen).

Methods: The parameters for IMS including specificity/sensitivity, bacterial concentration and bead incubation time were optimized. Limit of detection (LOD) was also determined for the optimized PCR-IMS and PCR-cen. Both methods were compared against PCR alone (PCR) and the standard culture method (ISO) for their ability to detect *Salmonella* using inoculated and uninoculated sprouts. Mean values were compared using ANOVA.

Results: Under optimum IMS conditions (10^5 CFU/ml for 30 min), capture efficiency of *Salmonella* in sprout suspensions was lower than 40%, most probably due to the non-specific binding of the background microbiota. PCR-IMS and PCR-cen had a similar LOD at 10^3 CFU/ml, which was one log unit lower than PCR. Enrichment of 10 h was sufficient to detect 100% of the inoculated sprouts with both PCR-IMS and PCR-cen, which was significantly faster compared to PCR and the ISO method. Moreover, the validation study using uninoculated sprouts revealed that PCR-IMS and PCR-cen were equally effective on *Salmonella* detection, showing 98.3% accuracy.

Significance: These results suggest that PCR-cen would be the effective and less costly method for the detection of low levels of healthy and sanitizer-injured *Salmonella* on mung bean sprouts.

T1-09 Whole Genome Sequencing-Based Identification and Comparative Analysis of Major and Putative Virulence Genes of *Escherichia coli* O103 of Bovine Fecal Origin

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❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* serogroup O103 is a common foodborne pathogen that can present as both potentially deadly Enterohemorrhagic *E. coli* (EHEC) and, less severely, Enteropathogenic *E. coli* (EPEC). Potential differences in disease outcomes, particularly serious complications, can be attributed to the diverse make-up of major and putative *E. coli* O103 virulence factors. Whole genome sequencing (WGS) has been used to analyze and characterize phylogenic relationships, virulence, and antimicrobial resistance gene profiles of EHEC, particularly of *E. coli* O157:H7. However, genome variations including those that encode for virulence genes of O103 serogroup are less characterized.

Purpose: Our objective was to utilize WGS to identify and compare major and putative virulence genes of EHEC and EPEC O103 isolates of bovine fecal origin.

Methods: A total of 69 O103 strains, previously identified by PCR as positive for *stx*1 (Shiga-toxin 1) and *eae* (intimin) (EHEC; *n*=43), negative for *stx*1 and positive for *eae* (EPEC; *n*=13) and negative for both *stx*1 and *eae* (*n*=13), were sequenced using WGS (Illumina MiSeq). Virulence genes, common and unique to O103 isolates, were identified using Virulence Finder 1.5.

Results: All EHEC and a majority (92.3%) of non-EHEC strains were O103:H2 serotype. The following genes differed between the EHEC and EPEC strains: EHEC adherence factor (*efa*1; 81.4 vs 7.7%), non-LEE encoded effector A (*nle*A, 100 vs. 53.8%) and C (53.5 vs. 0%), catalase peroxidase (*kat*P2; 67.4 vs. 7.7%), type III secretion system effector (*esp*J; 88.4 vs. 7.7%), and EAST-1 heat-stable toxin (*ast*A; 0 vs. 76.9%). Strains negative for *stx*1 and *eae* were negative for all above-mentioned virulence genes.

Significance: The WGS data indicate that bovine strains of O103 are diverse, but key gene patterns evolutionarily differentiate O103 EHEC from EPEC. The information can be used to increase the specificity of detection methods for risk assessment.

T1-10 NeoSeek *Salmonella*: A Rapid *Salmonella* Serotyping Platform via Next-Generation Sequencing

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Introduction: Antibody based serotyping is the most common form of sub-typing for *Salmonella* among diagnostic laboratories, public health entities and regulatory agencies. This Next-Generation Sequencing-based service produces definitive, gene-driven serotypes for isolates in 72 hours while removing the subjective interpretation of traditional methods. The method combines the throughput of NGS-based targeted amplicon sequencing with bioinformatics pipelines developed by MetaGenome Analytics. Targets for the assay were developed in collaboration with Dr. Jean Guard (USDA-ARS).

Purpose: With 2,557 recognized serotypes, accurate *Salmonella* serotyping by traditional methods is complex, expensive, and time consuming. Moreover, the availability of validated antisera can pose challenges. To circumvent these issues, NeoSeek *Salmonella* was developed.

Methods: NeoSeek *Salmonella* serotyping utilizes targeted amplicon sequencing of four distinct regions within the genome of *Salmonella*. PCR products for these regions are generated and sequenced on a MiSeq instrument. Assembled sequences are compared to a curated database of *Salmonella* whole genome and individual target sequences from known serotypes. Serotype assignment uses a sophisticated scoring matrix and built-in target redundancy for serotype determination.

Results: The targeted regions were examined in a single-blind, in silico study. The markers and scoring algorithm yielded a precision of >0.93 (n=68 strains) with strains that included the CDC "Top 30" *Salmonella*, as well as over 70 of the most common serotypes. The assay was subsequently validated with a panel of known serotypes (n=96 strains), yielding a precision of >0.95. Additional in silico analysis of the NeoSeek *Salmonella* targets against our database estimated that it can accurately differentiate over 1,500 different *Salmonella* serotypes.

Significance: NeoSeek *Salmonella* accurately reports *Salmonella* serotypes for isolates based on targeted amplicon sequencing. This genetics based platform does not rely on antisera to deliver accurate results. The shorter time to result provides for faster response times and can lead to quicker interventions over current methods.

T1-11 Enrichment, Amplification, and Sequence-Based Typing (EAST) of Foodborne Pathogens

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Introduction: Detection of foodborne pathogens typically involves microbiological enrichment with subsequent isolation and identification of a pure culture. This is ideally followed by strain typing, which provides information critical to outbreak and source investigations. Pulsed-field gel electrophoresis (PFGE) has for several decades been the gold standard for strain typing. Nevertheless, its multiple limitations have encouraged development of alternative methods including, most recently, whole genome sequencing (WGS). Both PFGE and WGS are technically challenging and require a pure culture, which adds to cost and time-to-result.

Purpose: There is a need for a facile, rapid, and robust method for foodborne pathogen detection and typing. To this end, an enrichment, amplification, and sequence-based typing (EAST) approach was developed for STEC, *Salmonella enterica*, and *Listeria monocytogenes*.

Methods: The EAST method involves: (1) overnight enrichment from food samples and total DNA preparation, (2) amplification of polymorphic tandem repeat-containing loci with electrophoretic detection, and (3) DNA sequencing and analysis for strain typing.

Results: EAST required <72 h and provided strain resolution better than serotyping and, for some typing targets, exceeding PFGE. Evaluation with ground beef and turkey samples spiked with strains of *L. monocytogenes*, STEC, or *S. enterica* demonstrated sensitivity (inoculum of ≤1 CFU/g) and specificity (unique or nearly unique alleles relative to >300 NCBI database strains). When EAST was applied to unspiked retail chicken parts, 3 of 11 samples yielded *S. enterica*-specific PCR products with sequence identities to strains of serotypes Schwarzengrund, Montevideo, and Typhimurium.

Significance: EAST provides a timesaving and cost-effective approach for detecting and tracking specific strains of foodborne pathogens, and post-enrichment steps can be commercially outsourced to facilitate implementation.

T1-12 Electrochemical Detection of *Escherichia coli* in Aqueous Samples Using an Engineered Bacteriophage with β-galactosidase Gene

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❖ ***Developing Scientist Competitor***

Introduction: Electrochemical detection of bacteria offers the advantages of instant quantification of pathogen in complex samples with minimal equipment. Bacteriophage has the high specificity and quick reproducibility which allows for the rapid and sensitive detection of bacterial pathogen. The use of the engineered phage with an enzyme gene provided opportunities to achieve a more sensitive detection: (1) release of the intracellular enzyme from *Escherichia coli* (*E. coli)*; (2) overexpression of more enzyme in *E.coli* during the specific infection.

Purpose: An electrochemical method was developed for the detection of *Escherichia coli* (*E. coli*) using the engineered bacteriophage with β-galactosidase (β-gal) gene.

Methods: Here, the bacteriophage T7 which specifically targets *E. coli* BL21, was engineered to carry the β-gal gene. The bacteriophage after the bioengineering therefore has the ability to express β-gal during the infection of *E. coli*. The released and phage-induced β-gal was detected by electrochemical methods using 4-aminophenyl-β-galactopyranoside (PAPG) as a substrate. The β-gal catalyzed PAPG to an electroactive species p-aminophenol (PAP) which can be monitored on the electrode. The electrochemical signal is proportional to the concentration of *E. coli*.

Results: We demonstrated the application of our strategy in aqueous samples (drinking water and apple juice). Using this method, we were able to detect *E. coli* at the concentration of approximately 10^5 CFU/ml in drinking water (or apple juice) in 3 hours, and 10^2 CFU/ml after 7 hours of incubation.

Significance: This method provides a specific and sensitive detection for *E. coli* in aqueous samples. It has the potential to be extended to detect other bacteria using different specific bacteriophage engineered with other enzyme genes.

T2-01 Prevalence and Antibiotic Resistance Pattern of *Salmonella* Serovars in Integrated Crop-Livestock Farms and Their Products Sold in Local Markets

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Introduction: The mixed crop-livestock (MCL) and backyard farms are major contributors to organic food production, however foods from integrated MCL and backyard farms, including chicken, egg, and fresh produce, are potentially in greater risk with cross-contamination of enteric bacterial pathogens as they are grown in close proximity. *Salmonella enterica,* the most common foodborne pathogen which infects humans and a wide range of animal hosts, potentially contaminates these products.

Purpose: The purpose of this study was to investigate pre- and post-harvest levels *Salmonella* prevalence, serovar prevalence, and their antimicrobial resistance patterns in the MCL farming system environments and its products available in retail stores.

Methods: A total of 1,287 samples from conventional poultry farms/organic MCL farms and 1,377 samples from organic/conventional retail supermarkets or farmers markets were collected from Maryland and the DC metropolitan area. *Salmonella* was identified with biochemical tests and PCR assay, and the serovars was determined by molecular subtyping multiplex PCR. Antibiotic resistance of the isolates was determined with agar dilution method.

Results: Totally, 315 *Salmonella* isolates were recovered, with 17.44% and 5.88% (n=315), from MCL and conventional farms samples (P<0.001). At post-harvest level, the prevalence of *Salmonella* was 30.95%, 19.83%, and 8.38% in chicken meat (P<0.001) from farmers, organic, and conventional markets, respectively, and 16.81% and 6.06% in produce products (P<0.001) from farmers and organic markets, but none from conventional markets. From the isolated *Salmonella*, 34.50% was confirmed *Salmonella* Typhimurium, followed by *Salmonella* Heidelberg (10.86%) and *Salmonella* Enteritidis (9.90%). The overall multi-antibiotic resistance in recovered *Salmonella* was 23.81% versus 4.55% in conventional and MCL farms (P=0.004) and 66.67% versus 7.76% in conventional and farmers markets (P<0.001).

Significance: This study demonstrates the potential food safety risks associated with MCL systems in terms of *Salmonella* especially *Salmonella* Typhimurium contamination on produce products and poultry meat in farmers markets.

T2-02 Trends in Risk Factor Behaviors in Temporary Eating Establishments in North Carolina

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Introduction: Temporary eating establishments have increased in popularity and frequency throughout the United States over the past decade. These establishments face numerous food safety challenges, related to staff and infrastructure; however, little literature exists on the risk factors that occur most frequently in these establishments. The U.S. Food and Drug Administration (FDA) publishes a model food code to aid state and local health authorities in regulating retail facilities based on current literature. FDA established a protocol for conducting a non-regulatory retail food risk factor study to measure the occurrence of commonly identified risk factors known to contribute to foodborne illness outbreaks. A similar study was conducted to identify risk factors for food safety non-compliance in temporary eating establishments in North Carolina.

Purpose: This study assessed FDA food safety risk factors observed at temporary food establishments in North Carolina.

Methods: Risk factor data were collected during non-regulatory visits by local health officials. Data collectors observed and documented behaviors for 43 operational risk factors associated with foodborne illness at 59 establishments. Risk factors were divided into 13 categories. Descriptive statistics were generated by category and eating establishment to assess the extent of non-compliance.

Results: The majority of establishments (88%) were out of compliance for at least one risk factor. The mean rate of overall noncompliance was 14.5% of risk factors. A total of 19.7% of establishments were out of compliance with factors related to employee hygiene, while 24.5% were out of compliance related to proper holding temperature, and 14.3% engaged in practices that could result in cross-contamination. A total of 71% of establishments had a certified food protection manager on staff.

Significance: Given the increase in temporary eating establishments throughout the United States, identifying trends in food safety practices and behaviors is critical to developing intervention strategies and evaluating policy.

T2-03 Using Theory of Planned Behavior to Predict School Nutrition Employees' Intentions to Use a Thermometer for Temperature Control

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Introduction: The risk of large-scale foodborne outbreaks in schools is high given that greater than 247 million meals and snacks are served annually. The Food and Drug Administration identified temperature control as one risk factor in schools. Thus, temperature monitoring can impact the occurrence of foodborne illness. Ajzen's Theory of Planned Behavior (TpB) states that attitudes, subjective norms, and perceived behavioral controls can best predict a person's behavioral intention. The TpB can be used as a tool to motivate employees' behavior change after predicting behavioral intention.

Purpose: Identify school nutrition employees' attitudes, subjective norms, perceived behavioral controls, and behavioral intentions to use a thermometer for temperature control for food production.

Methods: A total of 3,850 questionnaires were mailed to 163 participating school districts and distributed to employees. The questionnaire used 31 questions to assess direct measures including attitudes, subjective norms, and perceived behavioral controls; indirect measures including four behavioral beliefs, nine normative beliefs, and five control beliefs; and respondents' demographics. Principle Axis Factor Analysis and Multiple Regression were used for data analysis.

Results: Analyses consisted of a total of 408 questionnaires. The direct measures of behavioral intentions indicated that school nutrition employees had very high intention to use a thermometer for temperature control (M=6.9±0.37), had positive attitudes (M=6.9±0.37), placed emphasis on their subjective norms (M=6.8±0.45), and perceived a high amount of control (M=6.5±1.06) when using a thermometer. Perceived behavioral controls and subjective norms significantly predicted behavioral intention (P≤0.001), however attitude did not (P=0.463).

Significance: Since significant beliefs have been identified, interventions should be developed and/or modified to increase thermometer use in schools and improve employees' behaviors for temperature control to prevent foodborne illness. By focusing on perceived social pressures to engage or not engage in the behavior and behavioral controls to perform the practice, research opportunities exist for training effectiveness.

T2-04 Food Safety Challenges in Consumer Food Products at Hypermarkets in Pakistan

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❖ ***Developing Scientist Competitor***

Introduction: Changing life style and advent of hypermarkets have revolutionized the purchasing decision and eating habits of consumers around the globe. No doubt wide range of food products under one roof has reduced time pressure but food supply has raised many concerns about safety of food products sold in hypermarkets.

Purpose: The present study was planned to assess safety and quality of multiple food products available under one roof with huge diversity and to evaluate the perishability, traceability, packaging material, transportation and supply chain management practices to assure farm to fork approach.

Methods: Survey study was conducted through structured questionnaire at hypermarkets to identify food safety issues regarding supply chain, transportation, traceability, packaging material and perishability of food products. To determine the incidence of microbial load and some pathogens in perishable food products, a total of 170 sample (48 meat, 65 chicken sandwiches burger and 57 butter cream pastries) were aseptically collected from different display points, transportation vehicles and suppliers. Microbial analysis (TPC, Coliform, *E. coli*, *Staphylococcus*, *Salmonella* and yeast and mold) was done according to standard protocol of FDA within 4 hours of collection in microbiology lab, University of Veterinary and Animal Sciences, Lahore-Pakistan.

Results: To ensure traceability of food products was observed as major problem followed by inadequate and poor supply chain management practices especially unhygienic conditions of food carrying vehicles. No severe microbial proliferation was noted in the present study. Overall, the microbial status of food products was slightly good with 77.86% samples yielding acceptable results. While 10.65% samples were found unsatisfactory and 3.27% samples were potentially hazardous. Presence of *Salmonella* and *Staphylococcus* in chicken sandwich burgers indicated an alarming situation for final consumers.

Significance: The research findings will help quality department of hypermarkets, food suppliers, food transporters and food regulatory authorities to identify possible sources of contamination and take appropriate actions to ensure availability of safe food products in the market.

T2-05 Observed Food-Handling Practices among Adults Preparing Food during a Football Tailgate

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Introduction: Although there are numerous potential barriers to practicing safe food handling at outdoor locations, very little is known about the food handling practices of adults preparing food during college football tailgates.

Purpose: The purpose of this study was to observe and document the food handling behaviors of adults preparing food at football tailgates.

Methods: An observation checklist based on the clean, separate, cook, and chill concept was developed using input from food safety experts. Using this checklist, trained research assistants documented the food handling practices of adult participants (N=65). Observations occurred at two university locations and took place during six different games October through December 2015.

Results: The majority of participants were male (75.4%), greater than 35 years of age (58.5%), married (69.2%), and college graduates (73.8%). More than one-half reported that they prepared food daily (58.5%), and 41.5% indicated that one or more individuals at their tailgate were from a high-risk category (e.g., pregnant, under the age of 5). Observers noted that at 43.1% of the tailgate sites, neither hand washing nor sanitizing supplies were available. Of those with hand washing and/or hand sanitizing suppliers available, 24.3% of the participants never use the supplies, and 22.2% failed to use the supplies after every occurrence of contact with raw meat/poultry. Most participants took some measures to keep raw meat/poultry chilled (75.4%) and to keep raw meat/poultry separate from other foods stored in their coolers (67.7%). However, very few (10.7%) used a thermometer or temperature indicator to check the internal temperature of the food they were preparing.

Significance: Observation of food preparation revealed the common occurrence of practices that increase the risk of foodborne illness. Educational interventions targeted at this group are needed to decrease the risk of foodborne illness from food prepared at college football tailgates.

T2-06 Food Safety Knowledge at Kwazulu-Natal South Africa Households and the Microbiological Quality of Their Ready-to-Eat Foods and Food Contact Surfaces

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Introduction: Previous research has identified a huge gap in food safety knowledge at household level. This portends a risk for the possible occurrence of foodborne illnesses.

Purpose: This study determined the level of food safety knowledge and practices during food handling and preparation at household level in Kwazulu-Natal, South Africa and also examined selected foods (50 samples) and contact surfaces (50 samples) from households for the presence of aerobic bacteria, aerobic spore formers, anaerobic spore formers, *S. aureus, E. coli, Salmonella* spp. and *L. monocytogenes.*

Methods: Fifty (50) households were selected to participate based on their lifestyles, which was based on monthly income, age and educational level. Questions were asked to determine whether participants have knowledge on meat storage, thawing of meat, handling of meat among other attributes. Conventional methods including enrichment were used to determine aerobic bacteria, aerobic spore formers, anaerobic spore formers, *S. aureus, E. coli, Salmonella* spp. and *L. monocytogenes* in samples however *Salmonella* spp and *Listeria monocytogenes* were validated using the 3M Molecular Detection System.

Results: The results of the survey show that the majority of respondents generally relate food safety with food quality and nutrition. However, the study did reveal that a majority of respondents lacked food safety knowledge, although they did show an interest in learning more about good food safety practice. High counts of micro-organisms, including pathogens, were detected on contact surfaces and on the food that was to be consumed. For example all the selected foods were contaminated with *L. monocytogenes, Salmonella* and *E. coli* while almost similar trend was observed with contact surfaces although *L. monocytogenes* and *Salmonella* spp. were not isolated from plates and cutting boards, respectively.

Significance: This poses a health risk to consumers who eat food that has been incorrectly stored under conditions that afford a high risk of contamination.

T2-07 Influence of Sugars, Sanitizer, and *Lactobacillus rhamnosus GG* on Biofilm Formation of *Aspergillus* Species from Selected Meat Markets and Abattoirs in Ibadan, Nigeria

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Introduction: The ability of *Aspergillus spp.* to cause diseases in both human and animal population is of significance to public health. Furthermore, their biofilm forming abilities makes their control or treatment with some routine sanitizers or antifungal agents more complicated.

Purpose: This study investigated fungal contamination in meat markets and abattoir environments in Ibadan, Nigeria and different measures of biofilm control.

Methods: Table scrapings and swabs from four meat markets and three abattoirs were assessed for fungi counts (FC) and the prevalence of *Aspergillus species* using standard methods. Furthermore the influence of sucrose and fructose at 0.2% and 0.4% concentrations; Sodium hypochlorite (NaOCl) at 0.05% and 0.5% concentrations, and *Lactobacillus rhamnosus GG (LGG)* at 10^8 and 10^4 concentrations on biofilm development by *Aspergillus niger* and *Aspergillus flavus* was assessed. Biofilm was assayed on microtitre plates in Sabouraud Dextrose Broth. Modified Coconut Cream agar was used to assess the action of *LGG* on aflatoxin production by *A. flavus.*

Results: There were significant differences (P<0.05) in the FC among locations. *A. niger* (30.0%) was the most frequently isolated fungi, while the least was *A. fumigatus* (1.0%). There was a significant increase in biofilm mass with the addition of sucrose or fructose, with 0.2% concentration producing more biofilm mass (P<0.05). *LGG* produced a significant decrease in biofilm biomass for both species of *Aspergillus* tested (P<0.05). However, *A. flavus* significantly produced a higher biofilm mass than *A. niger.* There was reduced biofilm mass with both 0.05% and 0.5% NaOCl (P<0.05). Lower biofilm mass was formed at 11°C when compared to 28°C (P<0.05).

Significance: These results indicate that meat producing areas of Ibadan may be important sources of *Aspergillus* contamination of beef for human consumption. The presence of sucrose or fructose will enhance their biofilm forming abilities. *LGG* and NaOCl inhibit *Aspergillus* biofilm significantly at lower temperature.

T2-08 De Novo Assembly and Comparative Sequence Analysis of *Cyclospora cayetanensis* Apicoplast Genomes Originating from Diverse Geographical Regions

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Introduction: *Cyclospora cayetanensis* is a coccidian parasite causing foodborne and waterborne disease called cyclosporiasis. Since the 1990s outbreaks of cyclosporiasis occur almost every year in the U.S. and large outbreaks sickened hundreds of persons yearly from 2013 to 2015. Outbreak investigations are currently hampered because no molecular epidemiological tools are available for traceback analysis.

Purpose: The apicoplast is a non-photosynthetic plastid with an independent genome found in most apicomplexan parasites including *C. cayetanensis.* Genetic markers identified in apicoplast genomes of other parasites have been useful for detection and traceback analysis. Distinct differences in the apicoplast genomes of *C. cayetanensis* could be potentially useful to design advanced rapid molecular methods for rapid detection, subtyping and geographical source attribution, being applicable to outbreak investigations and surveillance.

Methods: We sequenced the *C. cayetanensis* genomic DNA extracted from stool samples from patients with cyclosporiasis using the Illumina MiSeq platform. Bioinformatic workflow included tools like Mulan, Bowtie2, Geneious, CLC workbench, RATT, MAKER2, and NCBI Blast++. The draft genome was manually curated using NGS data from *C. cayetanensis* in our collections. Raw reads from many samples originated from Nepal, New York, Texas, Indonesia and elsewhere were mapped to the apicoplast reference. Multiple alignment of apicoplast genomes with the reference assembly was carried out using MEGA6.

Results: Comparative analysis using curated and annotated circular 34146 bp reference genome resulted in assembly 20+ SNPs and some small indels spanning the reference genome, and a 31 bp-sequence repeat at the terminal spacer region unique to some Nepalese samples. Phylogenetic analysis of apicoplast genomes from *C. cayetanensis* displayed a familiar pattern of tight clustering with *Eimeria.*

Significance: This is the first report of end-sequence curated and annotated complete reference genome for the *C. cayetanensis* apicoplast. SNPs and sequence-repeats from this study can be used as genetic markers for geographic differentiation applicable to traceback investigations.

T2-09 Edible DNA Barcode Labeling for Authenticity and Traceability of Foods

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Introduction: For the food industry, traceability is a critical issue throughout all stages of production, processing, and distribution. Most food producers go through significant effort and expense to provide products that are easily traced back to their source. However, other producers use intentional mislabeling to distribute inferior products. This damages markets and creates an elevated safety risk, because the fraudulent products are difficult to trace. The key to advancing global food supply chain efficiency, accountability, and security is through traceability.

Purpose: We have developed a technology for enhanced traceability and monitoring of adulteration by labeling foods with unique and edible DNA "barcodes."

Methods: Synthetic DNA was mixed with carnauba wax and applied to fresh apples using a standard industrial coating process in a commercial packing facility. Similarly, cantaloupes were labeled with either one or both of two DNA barcodes (or none, as a control) in spiked carnauba wax through manual spraying in a commercial packing facility. Olive oil was also labeled with barcode DNA to gauge the ability of our technology to detect adulteration (by dilution), to evaluate the effects of edible DNA barcode on sensory qualities, and to assess the effects of olive maturity on DNA stability. Subsequently, DNA was extracted from the fruit wax coatings and olive oil, and qPCR was performed to measure recovery of barcode DNA. All samples were tested in triplicate.

Results: DNA was recovered from the surface wax of all 23 treated apples tested. DNA was also recovered from the waxed rinds of all 76 treated cantaloupes tested, with one false positive. Maturity level of olive oil had no measurable effect on DNA stability, and sensory testing showed no difference between tagged and untagged olive oil (P>0.05).

Significance: These results suggest that small amounts of DNA may be applied to food products using conventional processes, to facilitate traceability throughout the supply chain by distinguishing products specific to a producer and source.

Technical

T2-10 Beef, Buffalo and Pork Detection In Food Chain Using Double Gene-targeted Multiplex PCR Assay

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Introduction: Identification of animal species in food products has become an important and routine work under the supervision framework of most countries to prevent diseases transmitted by meat and bone meal as well as meat adulteration by mixing materials of different animal origins. Specifically, adulteration of porcine, bovine and buffalo material are of serious religious, health and economic concerns. Multiplex polymerase chain reaction (PCR) assays are greatly promising for the screening of multiple species targets in a single assay platform, saving analytical cost and time.

Purpose: The aim of the study was to develop double gene targeted short amplicon-length multiplex PCR assay for the confirm detection and differentiation of beef, buffalo and pork in raw and processed food products. Compare to a single target assay, targeting double gene sites must enhance the assay reliability through a complementation approach since it is unlikely that both gene targets will be missing under the food processing treatments.

Methods: Six different sets of primers, two for the each of beef, buffalo and pig, were developed targeting multicopy mitochondrial cytochrome b (cytb) and ND5 gene after the screening of specificity through bioinformatic software, mismatch analysis, 3D plotting and phylogenetic analysis. The final specificity was confirmed through PCR analysis against 20 different meat species followed by separation using automated capillary electrophoresis.

Results: All the six targets of length between 73 and 146 bp appeared both in the gel-image and electrpherogram both under raw and processed states of pure and complex food matrices of burger, meatball and frankfurter formulations.

Significance: It was the first report of a double gene targeted multiplex PCR assay for the confirmed detection of beef, buffalo and pork in food chain. The method would cut down the detection cost of the said species in food forensic or any archaeological investigation by at least three folds.

T2-11 Understanding False Positives in Mapping of Microbiome Sequence Data Using In-silico Simulations

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Introduction: Consider the task of mapping short read sequencing data of multiple genomes in a micro-environment to the correct organism or, more generally "Operational Taxonomic Unit" (OTU), using a reference database. Potential challenges here include reference databases having redundant candidates or lacking sufficient accuracy; many different species or strains in the environment being genetically very close leading to confusion; the extraction process and associated biotechnology introducing sequencing errors. These factors confound the mapping problem, leading to inaccurate results and subsequent interpretations.

Purpose: Most existing solution pipelines yield read mapping results that are riddled with false positives. For instance, through in-silico simulations, we find that up to 85 to 90% of the predicted potential OTU set obtained using standard pipelines from literature are false. We tackle this problem by introducing a computational solution.

Methods: Our method is based on promiscuity of reads, i.e., reads mapping to multiple OTUs, in contrast to current approaches that rely on the abundance of reads. Ranking the potential OTU matches for each read, we demonstrate through simulations that the rank frequency distribution of true positive OTUs' reads peak at rank 1. To further enrich the true positives, we define a normalized score per OTU, based on the promiscuity. Sorting by the score, the false positive OTUs sink to the bottom.

Results: Our preliminary experiments demonstrate that false positive OTUs can be substantially reduced, without losing any true positives. Using wgsim we simulated 10,000 sequencing reads of 100 bp from the 16S genes of 20 bacterial species, including food pathogens, from 13 genera. Averaging the results over 100 instances we obtain the following: the method reduced an average of 368 false positive OTUs down to a mere 29, without losing any true positive in any of the instances.

Significance: More accurately identifying the truly present organisms in food samples benefits downstream analyses including hazard detection.

T2-12 Withdrawn

T3-01 Multi-Criteria Decision Analysis for Risk Management of Microbial Hazards in Low-moisture Foods

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Introduction: Following a number of recalls and outbreaks, there has been increasing international interest in the microbial safety of low moisture foods (LMF) such as dried herbs and spices, chocolate, raw almonds, sesame, peanut butter, breakfast cereals, and more. The most commonly associated hazards are *Salmonella*, *Bacillus cereus*, *Clostridium botulinum*, *C. perfringens*, pathogenic *Escherichia coli*, and *Staphylococcus aureus*.

Purpose: Towards developing a Draft Code of Hygienic Practice for LMF, the Codex Committee on Food Hygiene (CCFH) asked the FAO and WHO to provide advice on which foods should be the highest priorities and on risk management options. FAO and WHO initiated a series of activities to provide guidance, including knowledge synthesis activities, and culminating in a 3-day expert workshop in May 2014.

Methods: A multi-criteria decision analysis (MCDA) model was developed interactively with subject matter experts over 3 days to rank seven LMF product categories based on a number of criteria relating to public health and international trade. Swing weights were elicited from experts during the meeting, sensitivity analyses were conducted, and further data collection was used to parameterize attributes. The ranking was finalized interactively with experts via web-conference following the meeting.

Results: Expert-selected model attributes related to disease burden, pathogen contamination, food production characteristics, annual trade impacts, food consumption patterns, and population vulnerabilities. Cereals and Grains scored highest overall, and particularly for international trade and food consumption criteria. Dried protein products ranked second and stood out in terms of disease burden due to a couple of very large outbreaks associated with dried dairy products. Dried spices and herbs ranked third and had high criteria values associated with food production characteristics and outbreak disease burden.

Significance: The MCDA ranking, risk management recommendations, and related materials were presented to CCFH and guided discussion in determining priorities for future risk management efforts.

T3-02 Development of a Probability Model to Describe the Uncertainty of the Time to Inactivation of *Salmonella enterica* under a Desiccated Environment

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Introduction: During the process of bacterial cell inactivation, frequency distribution of the time to inactivation occurs due to the probabilistic nature of the event. The variability of bacterial survival is apparently observed in a small population. Less than 100 viable cells of *Salmonella enterica* are sufficient for the infection of humans. Estimating the time to inactivation probabilistically is useful to determine a realistic estimation of the risk of the pathogenic bacteria. We focused on low water activity foods as critical pathogen carriers.

Purpose: We aimed to develop a predictive model to describe the variability of the time to inactivation of *Salmonella enterica* Typhimurium under a desiccated environment through a bacterial experiment and a computer simulation.

Methods: In the bacterial experiment, aliquots of 2 µl of a *Salmonella* Typhimurium suspension were placed into a 96-well microplate, and the microplate was then placed in a drying chamber (10-20% RH) at different temperatures ranging from 5°C to 25°C. The survival of the cells in each well was confirmed by the turbidity after adding nutrient broth (100 µl). In computer simulation, the time of inactivation of the bacterial populations was estimated by random sampling from individual cells in the kinetics model.

Results: The survival probability was estimated by the number of the surviving wells in the 96-well microplate and was described as a cumulative gamma distribution. In addition, the variability of the time to inactivation of bacteria was described as a gamma distribution. The larger the initial bacterial numbers are, the wider the range of time to inactivation observed in both the experiment and the computer simulation.

Significance: The probability model could be used for describing the variability of the time to inactivation of bacterial population, which would play an important role in assessing the risk of low water activity foods.

T3-03 Identification of Two Virulence Genes Involved in *Salmonella*'s Ability to Survive Desiccation

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Introduction: *Salmonella* is responsible for the largest number of outbreak-related hospitalizations. Many outbreaks have been associated with dry foods due to the unique ability of *Salmonella* to survive in low water activity.

Purpose: The objective of this study was to investigate the molecular and physiological mechanisms of cells exposed to low water activity.

Methods: Total RNA-Seq was used to determine transcriptional changes of *Salmonella enterica* ser. Typhimurium inoculated on filters and equilibrated at a_w 0.11 and control a_w (a_w 1.0). KEGG Orthology classification was performed and knock-out mutants of genes of interest were created to assess their role on *Salmonella*'s ability to survive desiccation. Wild-type (WT) and mutant strains were inoculated on micro glass-beads, dried, equilibrated to a_w 0.11 and 1.0 and enumerated, while phenotypical changes were compared by scanning electronic microscopy.

Results: The transcriptional analysis revealed 290 genes upregulated in cells at a_w 0.11 compared to 1.0, including two virulence genes: *sopD* and *sseD*. The viability of *sopD* and *sseD* mutants dried on beads was significantly lower than WT cells (≥1 log CFU, $P \leq 0.02$). After exposure to a_w 0.11 the differences in viability were greater than 2 log CFU between WT and mutants ($P<0.01$). Mutant cells had very distinct morphology from the WT with shorter and rounder cells. Micrographs of dried cells and exposed to a_w 0.11 depicted an evident indentation in the middle of the cell and the lack of solid exopolymeric substances, with only a fibrous and filamentous net present.

Significance: Understanding which cellular components and molecular regulators are involved in *Salmonella*'s ability to survive thermal processing on low a_w matrices is fundamental to combat persistence of this foodborne pathogen on low a_w matrices and its acquired thermal tolerance. This work discovered the connection of two genes with *Salmonella*'s survival to desiccation.

T3-04 Survival of *Salmonella enterica* and a Surrogate Microorganism, *Enterococcus faecium,* on Whole Black Peppercorns and Cumin Seeds Subjected to Ethylene Oxide Fumigation

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Introduction: In recent years, the microbiological safety and quality of spices has caused concern for regulators and the food industry. Further processing using the chemical fumigant ethylene oxide is performed for many spices to reduce microbial populations and ensure a safe product of high quality. Reports on the effectiveness of ethylene oxide fumigation against *Salmonella enterica* on whole spices are not readily available.

Purpose: The objective of this research was to examine the effectiveness of commercial treatment of whole black peppercorns and cumin seeds with ethylene oxide on the survival of *Salmonella enterica, Enterococcus faecium,* and total aerobic bacteria.

Methods: Whole black peppercorns and cumin seeds were inoculated with a *Salmonella* cocktail or *Enterococcus faecium* NRRL B-2345 using agar grown cells, and the spices were dried to a_w 0.3 to 0.5. Spices were packaged (5 lb) in polywoven bags (n=3) and shipped to a commercial processor for EtO treatment using FDA approved spice treatment parameters and returned by overnight shipment. Cells were enumerated by serial dilution and plating onto TSA for aerobic plate counts or TSA with a thin overlay of XLT4 for *Salmonella,* or BEA for *E. faecium*.

Results: Ethylene oxide fumigation significantly reduced the populations of *Salmonella* and *E. faecium* on whole black peppercorns and cumin seeds ($P<0.05$). Reductions of *Salmonella* on peppercorns (6.62±0.66 log CFU/g) were significantly greater than those for *Enterococcus* (P=0.05, 2.96±0.66 log CFU/g). Reductions of *Salmonella* and *Enterococcus* on cumin seeds were not significantly different (P=0.38; 4.9 and 4.3 log CFU/g, respectively). EtO fumigation significantly reduced total aerobic plate counts for both spices by 2 to 3 log CFU/g.

Significance: Ethylene oxide fumigation offers a strategy to reduce *Salmonella* on whole spices. Further research examining gas penetration needs to be performed to verify that the process is adequately inactivating *Salmonella* using all packaging configurations.

T3-05 An Assessment of Time/Temperature Combinations for *Salmonella* Lethality Achieved when Baking Cookies in Vehicles

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❖ ***Developing Scientist Competitor***

Introduction: Consuming raw or undercooked baked goods is a risk for foodborne illness and has been linked to over 175 illnesses associated with five outbreaks. Pathogens including *Salmonella* spp. and *E. coli* O157:H7 have been associated with these products. The media has popularized attempts to bake cookies inside a vehicle on hot, sunny days, assuming a car simulates an oven without knowledge of safety.

Purpose: No data is currently available on the time/temperature profiles of a low-moisture raw cookie product during car baking and the safety of the final products. The goal was to see if vehicle-baked cookies reach a safe time/temperature combination.

Methods: The internal temperatures of car-baked cookies (*n*=8, 5 replicates) were measured during the summer of 2014 in North Carolina using two types of vehicles and two types of cookies. A data logger inserted into each of eight cookies, two of each type per vehicle, monitored temperature in five-minute intervals. Two additional data loggers monitored internal temperatures of each vehicle. Qualitative observations were taken at regular intervals and the experiment was repeated on five separate days of varying outside temperature.

Results: Based on the literature, a 3-log reduction of *Salmonella* can be achieved at 65°C for 10 minutes or 74°C for 1.5 minutes when water activity is 0.82. All cookies achieved a safe time/temperature combination within four hours. Maximum internal temperatures ranged from 65.5°C to 88.6°C. Fifty-eight percent (*n*=36) reached 74°C in an average of 61 minutes and 39% reached 65°C in an average of 76 minutes. Cookies did not brown, were softer than traditional oven-baking and took an average of 183 minutes to appear set.

Significance: This experiment shows with average daytime temperature highs of 33°C (n=5), it is possible to bake cookies in a vehicle and achieve a 3-log reduction of *Salmonella spp.* within two hours.

T3-06 Isothermal Inactivation of *Salmonella* and *Enterococcus faecium* in Dates Impacted by Water Activity Variation at Elevated Temperature

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Introduction: Temperature and water activity (a_w) are highly affecting the thermal inactivation efficiency of microorganisms in low-moisture foods; however, the influence of a_w at elevated temperatures on thermal resistance of *Salmonella* and its surrogate *Enterococcus faecium (E. faecium)* in dates is not yet available.

Purpose: (1) To compare thermal resistance of *E. faecium* with a *Salmonella* Enteritidis PT30 (*Salmonella* PT30) on date surfaces in the same thermal treatments, and (2) to understand the relationship between temperature-induced changes in a_w and thermal resistance of *Salmonella*.

Methods: Dates were inoculated with *E. faecium* or *Salmonella* PT30 and equilibrated to different $a_{w,\ 25°C}$ (0.30, 0.45 and 0.60±0.02) to establish 8.5±0.1 CFU/piece. The samples were vacuum-sealed in thin plastic bags, heated isothermally at 75, 80 and 85°C, cooled after treatment, and enumerated to obtain *D*-values. Equilibrium water sorption isotherms (moisture content vs. a_w) for dates at 20 to 80ºC were generated using a vapor sorption analyzer and a thermal cell with relative humidity sensor, and compared using ANOVA.

Results: *E. faecium* showed equal or higher thermal resistance than *Salmonella* PT30 at all a_w levels (e.g., at $a_{w,25°C}$=0.45±0.02, 80°C, $D_{E.\ faecium}$=4.22±0.72 min, $D_{Salmonella\ PT30}$ =2.60±0.42 min). A flat isotherm curve was observed for dates below $a_{w,25°C}$=0.5, indicating constant moisture content and same a_w at elevated temperatures. It may explain the statistically same $D_{85°C}$ with different initial a_w (e.g. $D_{E.\ faecium,\ aw,25°C=0.30}$=1.64±0.86 min, and $D_{E.\ faecium,\ aw,25°C=0.45}$=1.60±0.80 min).

Significance: Improved understanding of the relationship between temperature-induced changes in a_w of dates and the thermal resistance of *Salmonella* and *E. faecium* is critically important in designing and validating thermal processing for ensuring dates' safety.

T3-07 Effect of Radio Frequency on the Reduction of *Enterococcus faecium* in Raw Peanuts

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Introduction: Multiple outbreaks of salmonellosis have been associated with the consumption of low-moisture products, including peanuts and peanut butter. *Enterococcus faecium* has already been used in the evaluation of oil and dry roasting processes of peanuts as a surrogate for *Salmonella*. Alternative technologies like radio frequency (RF) are being used for nut roasting and shelled almond pasteurization. Therefore, scientific information on the effect of RF processing on *E. faecium* reduction in raw shelled peanuts treated is needed.

Purpose: The goal of this research was to determine the level of *E. faecium* NRRL B-2354 reduction in runner-type peanuts treated with RF technology.

Methods: Product (1,000 g) was inoculated (~7 log CFU/g) with *E. faecium* NRRL B-2354 using dry inoculation. Inoculated peanuts were packed in 25 g aliquot in nylon bags. A box (11 lbs) holding three bags, located in the coldest area, was placed at center of the cavity of the RF system and treated until the target temperature was achieved. Peanuts were RF treated (27 MHz) at 3 temperatures, ≥85°C, ≥110°C and ≥126°C, with three different process times. Each temperature and time was evaluated in triplicate. After each treatment, the pouches were immediately cooled in ice and plated.

Results: RF treatment produced <2.0 log CFU/g reduction of *E. faecium* at ≥85°C, and ≥5.0 log CFU/g reduction at ≥110°C and ≥126°C.

Significance: The significance of this study is that a 5 log CFU/g reduction of *E. faecium* in raw peanuts with RF processing was achieved at temperatures lower than oil (120 to 160°C) and dry (129 to 163°C) roasting.

T3-08 Contaminations of the Food Supply Chain: Rapid Targeting of Sources with Modern Data Analytics

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❖ ***Developing Scientist Competitor***

Introduction: Determining the spatial origin of a contaminated food causing an outbreak of foodborne disease is a challenging problem due to the complexity of the food supply and the absence of coherent labeling and distribution records. Current investigative methods are time and resource

intensive, and often unsuccessful. New tools and approaches that take advantage of modern data and analytics are needed to more quickly identify outbreak origins and prioritize response efforts.

Purpose: The aim of this research was to develop a methodology to efficiently identify the source of an outbreak while contamination-caused illnesses are still occurring, thereby resolving investigations earlier and averting potential illnesses.

Methods: A network-theoretical approach was developed for rapid identification of the source of foodborne contamination events. Given the spread of outbreak-related cases and limited data on the distribution network, the inference approach uses backward induction and network analysis to determine the probability that any location is the outbreak source; the greater the number and dispersion of cases, the fewer locations are suspect due to the topological properties of the network. A probabilistic simulation approach involving distribution network models of selected food products was developed to model the accuracy and robustness of the approach across multiple outbreak scenarios.

Results: In extensive simulation testing across a variety of realistic distribution network structures, the outbreak source is robustly ranked within the top 5% (1%) of feasible locations after 5% (25%) of the cases have been reported, reducing by up to 45% (25%) the eventual total number of illnesses in the simulated outbreaks. The method greatly outperforms all heuristics, which can be viewed as representative of investigation strategies applied in practice.

Significance: Our results suggest this methodology that can form the basis of a "tool" to supplement existing traceback processes, enabling regulators to identify high probability sources of an ongoing outbreak, and to make strategic recommendations regarding allocation of investigative resources and search effort.

T3-09 Burden of Disease of Dietary Exposure to Acrylamide in Denmark

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❖ ***Developing Scientist Competitor***

Introduction: Acrylamide (AA) is a process-contaminant that potentially increases the risk of developing cancer in humans. AA is formed during heat treatment of starchy foods and is detected in commonly consumed products such as breakfast cereals, french-fries and coffee. Due to its potential health impact, AA and has received much attention in the last decade.

Purpose: Increased focus on risk ranking and prioritization of major causes of disease makes it relevant to estimate the impact that exposure to chemical contaminants in foods have on health. We estimated the burden of disease caused by dietary exposure to AA, using disability adjusted life years (DALY) as health metric.

Methods: We focused on cancer as health outcome and proposed a burden of disease model of three components: an exposure-, health-outcome-, and DALY-module. To evaluate the impact of modelling choices in the model components, we estimated disease burden using two approaches for estimating incidence of disease based on quantitative toxicological data, and two approaches for estimating DALY.

Results: Exposure to AA in foods causes 0.18 cancer cases per 100,000 inhabitants and a burden of 1.8 DALY per 100,000 in Denmark each year as estimated by the most conservative approach. We attributed 45-48% of this disease burden to the exposure of AA through intake of fried potato products.

Significance: These results are useful to inform risk management decisions and for comparison with burden of disease of other food-borne hazards. Our study also shows that careful evaluation of methodological choices and assumptions used in burden of disease studies is necessary before use in policy making.

T3-10 Development of a Predictive Tool for Assessing Vulnerability to Economically Motivated Adulteration

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Introduction: Economically motivated adulteration (EMA) is an established threat to grocery manufacturers. In today's globally distributed and dynamic food supply network there are inherent risks to the integrity of the supply chain. The impact of EMA can range from minor (e.g., no public health impact and minor economic damage to an organization) to catastrophic (e.g., loss of life and loss of economic viability of an entire organization). Current methods of assessing potential vulnerabilities to EMA are reactive in nature and are unable to help prioritize decisions across the wide array of commodities many food safety and defense professionals must manage on a day-to-day basis.

Purpose: The purpose of this presentation is to demonstrate a predictive, software-based tool for assessing the vulnerability of commodities to EMA based on a combination of characteristic attributes and subject matter expert (SME) opinion.

Methods: A cloud-based software tool has been created that estimates the vulnerability of commodities to EMA by combining automated data mining of commodity characteristics (value, volume, scarcity, etc.), a multi-attribute utility model algorithm, and SME opinion.

Results: The development of the foundation for assessing EMA vulnerability will be discussed, including: (1) the selection, quantification, and mining of characteristic attributes will be discussed; (2) the incorporation of mathematical algorithms based on utility modeling to capture EMA perpetrator hesitancies; and (3) SME opinion regarding the relative importance of commodity characteristics in driving EMA perpetrator behavior. The resulting tool provides critical insight on EMA vulnerability that provides a defensible, consistent assessment able to inform decisions. Retrospective case studies that demonstrate application of the EMA Vulnerability Assessment Tool will also be presented and discussed.

Significance: This presentation provides a clear example of how a software-based decision aid can improve the efficiency and consistency of evaluating EMA vulnerability.

T3-11 Identification of Steps within Nodes of the Food Supply Chain which Could Facilitate a Foodborne Terrorist Attack

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Introduction: The Department of Homeland Security Chemical Security Analysis Center analyzes intentional contamination of the food supply as one of the six major targets in the Chemical Terrorism Risk Assessment. Identifying the points in the food supply at which a terror-inspired intentional contamination could affect the public health is the first step in mounting an effective yet rational defense. Within the many nodes that make up the supply chain, determining the likelihood of a successful mass contamination within a specific node is difficult. To date there has been no freely

available mechanism for assessing this likelihood based on agent characteristics, processing parameters and a numerical estimate of a motivated individual accessing the process.

Purpose: The purpose of this effort was to produce a tool which could be used by vetted Food & Agriculture Sector members to determine where within their process, the risk of an intentional contamination not only exists but exceeds a rational "threshold" of risk.

Methods: This work combines the industry perspective, *where does unacceptable risk exist within a process*, and the Government perspective, *what harm could be done*, into a science-based model which is intended to address needs at the facility and corporate levels of industry as well as the needs of those responsible for addressing public health concerns.

Results: The model evaluates the likelihood of a motivated aggressor being able to access the product and the likelihood agents will remain viable within the process to estimate the risk at each defined process step for each agent. Potential health effects of the intentional contamination at each process step are also estimated.

Significance: This effort will culminate in a downloadable tool accessible by stakeholders to analyze the risk within their specific nodes, focusing mitigation strategies where they are most needed, thus reducing risk and bolstering food defense.

T3-12 Impact of Roasting of Cocoa Nibs and Beans on Inactivation Kinetics of *Bacillus cereus* and *Geobacillus stearothermophilus*

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Introduction: *Bacillus cereus* (BC) is a pathogen that causes two types of syndromes (emetic and diarrheic). *Geobacillus stearothermophilus* (GB) is the causative agent of "flat sour" spoilage in low-acid thermally processed foods.

Purpose: To determine the inactivation kinetics of BC and GB on cocoa nibs and beans at 110, 125 and 140ºC.

Methods: Spores suspensions of five different strains of each BC and GB were used. Cocoa beans and nibs were put in sterile steel trays and separately inoculated with spores of BC and GB, respectively. The roasting process was carried out at three temperatures (110, 125 and 140 ± 2ºC). Samples were collected in different time periods and survivors were enumerated on MYP (BC) and DTA (GB). Data on survivors (CFU/g) were plotted against time and inactivation kinetic parameters were determined by fitting predictive models to data using the add-in Ginafit.

Results: The inactivation kinetics of BC and GB were estimated using Weibull-type models. The *δ*-values (time for the first decimal reduction; min) for BC in cocoa nibs at 110, 125 and 140ºC were 79.2±16.7, 47.9±1.2, and 24.1±0.1, whereas for cocoa beans these values were 166.2±24.1, 702±26.3, and 31.3±3.2, respectively. The *δ*-values (min) for GB in cocoa nibs at 110, 125 and 140ºC were 117.8±27.1, 212±0.4 and 69.9±13.5, while in cocoa beans these values were 278.2±66.0, 58.4±8.5 and 61.6±13.8, respectively. The *4D*-values (time for 4 log CFU reduction; min) for BC in cocoa nibs at 110, 125 and 140ºC were 281.1±7.3, 107.2±0.9 and 59.6±4.7; whereas, in cocoa beans, *4D*-values were 389.2.±9.5, 143.1±19.0 and 65.2±2.8. The *4D*-values for GB in cocoa nibs were 427.8±18.5, 153.0±4.2 and 137.6±6.8, while for cocoa beans were 564.3±23.3, 228.6±12.7 and 156.0±8.5.

Significance: These data suggest that the temperature conditions applied during cocoa roasting will not impact on BC and BG spores.

T4-01 Reduction of Tulane Virus (a Human Norovirus Surrogate) by Chlorine Dioxide (ClO_2) Gas

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❖ ***Developing Scientist Competitor***

Introduction: Human noroviruses (HNoV) are reportedly responsible for 400,000 emergency visits with $2 billion in financial losses yearly within the United States alone. Recently, Tulane virus (TV) has been used as a cultivable HNoV surrogate to determine effective inactivation strategies against HNoV. Chlorine dioxide (ClO_2) gas, a strong oxidizer has been used in water disinfection. The advantages of ClO_2 gas over solution include greater penetration ability that results in improved effectiveness. Recently, reduction of 3 log PFU/ml MNV-1 with 2.5 mg/L after 2 min and to non-detectable levels with 4 mg/L ClO_2 gas after 1 min were reported. Inactivation of TV by ClO_2 gas is unknown.

Purpose: The objective of this research was to determine the ability of ClO_2 gas to inactivate TV at room temperature (RT).

Methods: TV at ~6 log PFU was aseptically dried on sterile formica coupons and treated with 2.5 and 4 mg/L of ClO_2 gas for 30, 60, 120, and 300 s at ~75% relative humidity (RH) and RT. At each time point, TV was recovered using cell-culture media containing 10% fetal bovine serum. TV infectivity was assessed by plaque assays in duplicate using LLC-MK2 host cells. Data obtained from triplicate treatments were statistically analyzed.

Results: TV (~6 log PFU) was reduced by 1.16±0.25, 2.56±1.38, 3.37±1.67 log PFU and to non-detectable levels with 2.5 mg/L ClO_2 gas treatment after 30, 60, 120 and 300 s, respectively at ~75% RH and RT. Increased TV reduction of 3.36±1.77, 3.37±1.54, 4.59±1.13 log PFU and to non-detectable levels was obtained with 4 mg/L ClO_2gas treatment after 30, 60, 120 and 300 s, respectively, at ~75% RH and RT.

Significance: This study showed that ClO_2 gas treatments are effective in decreasing TV titers in a time- and concentration-dependent manner similar to MNV-1. Further studies on ClO_2 gas applications on produce are needed to prevent HNoV transmission/outbreaks.

T4-02 Development of Portable Electrochemical Sanitizing Unit Based on Diluted Sodium Chloride Solution for Generating Neutral Sanitizer

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Introduction: Currently many commercial sanitizers are not environmentally and consumer friendly. Affordable facility using natural harmlessness materials to generate effective sanitizers would be critical for being welcomed by consumers.

Purpose: The purpose of this study was to develop a portable and affordable sanitizing unit that can effectively control foodborne pathogens including *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

Methods: Oxidation–reduction potential (ORP) and pH of generated electrolyzed water were assayed by meters. Free available chlorine (FAC) was determined by a liquid kit. Partial catholyte solution was reintroduced to electrolysis for modifying the output electrolysis water to be pH neutral. A mathematical modeling was proposed to describe the input-output and optimization of the FAC production of this electrolysis system. Sanitizing effects of generated sanitizers from this unit on *E. coli* O157:H7 C7927 and *L. monocytogenes* BAA-839 were compared with electrolyzed water generated from a commercial large unit.

Results: A RuO2-IrO2/TiO2 PTFE electrode with dimension 10 cm × 5 cm × 1 cm was found very effective. Optimization equations were generated for FRC, pH and ORP, respectively. For a starting concentration of 8.29 ± 0.07 log CFU/ml of *E. coli* O157:H7, 4 mg/L of the two solutions generated from developed unit and a commercial large unit resulted in 5.70 ± 0.09 and 5.75 ± 0.23 log CFU/ml, respectively. Similar results were found for *L.*

monocytogenes. The developed sanitizer was pH neutral (7.08 ± 0.08) compared to the commercial one (3.77 ± 0.18). Moreover, the developed sanitizer had similar sanitizing effect on both *E. coli* O157:H7 and *L. monocytogenes* commercial sanitizer generated from large unit. At 40 mg/L, both sanitizers achieved ≥6 log CFU reductions (P>0.05).

Significance: The results suggest that the developed prototype unit is promising as a sanitizing unit for consumers to control foodborne pathogens.

T4-03 Resistance to Four Sanitizers of Different Strains of *Salmonella* and *L. monocytogenes* in Biofilms of Stainless Steel

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Introduction: Biofilms on stainless steel surfaces of food processing are extremely important as they may lead to food spoilage and occurrence of foodborne disease outbreaks. The persistence phenomenon can be attributed to an increased tolerance of microorganisms to sanitizers and further adhesion ability to abiotic surfaces.

Purpose: This study aimed to assess the resistance to sanitizers (sodium hypochlorite, chlorine dioxide, peracetic acid and quaternary ammonium) of different strains of *Salmonella* and *Listeria monocytogenes* when in biofilms.

Methods: Four strains of each, *Salmonella* and *L. monocytogenes*, previously screened for sanitizer's resistance were inoculated in a 12-well plate (1 strain/well) containing 3 ml of Luria Bertani broth and sterile stainless steel coupon (AISI #304, 10mm x 10mm x 1mm). The 12-well plates were further incubated at 37°C/24 h, following removal of coupons from wells and washing for 1 minute in 10 ml of PBS. Then, coupons were placed in contact with 3 ml of selected sanitizer (5, 10, 20, 25, 50 and 100 ppm) for 3 min. Next, the coupons were vortexed for 2 min to detach cells adhered to coupons, followed by drop plating in Muller Hinton Agar for enumeration of survivors (37°C/24 h).

Results: Quaternary ammonium (QAC) did not inactivate *Salmonella* in all tested concentrations, while chlorine dioxide (ClO_2) was the most efficient sanitizer. Reductions of adhered cells of *L. monocytogenes* 4b reached up to 7.1 log in treatment with ClO_2 at 20 ppm. Differences in terms of resistance in biofilms were clearly noticed among the different strains of *L. monocytogenes*. *L. monocytogenes* survivors ranged from 3-6.4 log reduction when a concentration of 10 ppm of ClO_2 was used.

Significance: The resistance of different strains of *Salmonella* and *L. monocytogenes* when in biofilms to different sanitizers. These results may assist the development and application of sanitization strategies to eliminate biofilms from food processing environments.

T4-04 Environmental and Product Related Factors that Influence Pulsed Light Inactivation of Foodborne Pathogens and their Surrogates in Foods

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Introduction: Pulsed Light (PL) treatment has the capability of inactivating foodborne pathogens, while maintaining unchanged many food quality parameters that are typically altered by thermal processes. Yet, the efficiency of PL treatment is affected by many substrate and environmental factors.

Purpose: The objective of this study was to investigate the effect of substrate related factors and light-substrate interactions on the efficacy of PL inactivation.

Methods: Liquid substrates ranged from transparent Butterfield's phosphate buffer (BPB), to apple juice, cider and milk of different composition. As challenge organisms, *Listeria monocytogenes* and its surrogate *L. innocua*, as well as *E. coli* O157:H7 and a nonpathogenic surrogate were used, at 10^7 to 10^9 CFU/mL. A thin layer of inoculated liquid sample, contained in either a glass chamber or a UV reflective aluminum dish, was treated with PL doses ranging from 0.4 to 14.9 J/cm^2. Survivors were recovered, and inactivation was quantified by standard plate counting. The effect of temperature on inactivation was also evaluated. Treatments were replicated and inactivation data was evaluated statistically.

Results: PL inactivation was diminished by sample turbidity and color, due to scattering and absorption of light by the sample. A 9-log reduction of *E. coli* was achieved in BPB, but only 2-log reduction in apple juice and cider, under similar conditions. Inactivation in apple juice was improved significantly by turbulence, and more than 5-log reduction was achieved under turbulent conditions. Inactivation was also improved by using an UV reflective sample holder. A statistically significant difference (P<0.05) was achieved between inactivation in non-UV reflective (6.41±0.32 log) vs. UV-reflective (7.83±0.41 log) containers. The temperature of the sample during treatment also affected inactivation.

Significance: This data demonstrates the potential of PL to inactivate bacterial pathogens in foods, while highlighting the importance of considering food properties and environmental conditions when designing PL applications.

T4-05 Combating *Listeria* at Ice Cream Facilities - A Case Study

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Introduction: One of the major stories in 2015 was of the *Listeria monocytogenes* contamination that hit the ice cream industry. This presentation aims to discuss the decontamination that took place at one such facility using chlorine dioxide gas.

Purpose: The purpose of the decontamination was to kill the *L. monocytogenes* that put a halt on production. *Listeria* is a somewhat common, and very serious problem within cold food processing areas. The facility chose to decontaminate the processing areas with chlorine dioxide gas, a proven sterilant capable of killing *L. monocytogenes*.

Methods: The chlorine dioxide gas decontamination took place over the course of two days. The first day consisted of the setup of all equipment, and the sealing of all doors and penetrations into the space. Injection lines were brought to various places throughout the facility as were concentration monitoring lines to measure the amount of chlorine dioxide gas in various locations. The second day consisted of the decontamination itself, as well as the removal of all equipment.

Results: The decontamination proved to be a success. Concentration monitoring data showed that all locations within the facility were exposed to the proper dosage required to exhibit a 6-log, sterilization level kill (99.9999% reduction) of all microorganisms including spores. Biological indicators (or spore strips) were placed throughout the facility to prove efficacy as well, with each of those confirming that a sterilization level kill took place. Finally, swab testing confirmed that the production area no longer contained *L. monocytogenes* as well.

Significance: Aside from the significance given to this issue from the media, and a significant hit to a company's financials, listeria contamination of cold food processing areas is of major concern to the industry. A method and process to eliminate it is of great significance to those working in those facilities, as listeriosis is a major health issue for consumers if contamination occurs.

Technical

T4-06 The Effect of Bacterial Diversity and Physicochemical Factors on the Survival of *Listeria monocytogenes* in Soil

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❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* (Lm) is the causative agent of many fresh produce outbreaks in recent years. Soil has been identified as one of the major risk factors for Lm contamination at the pre-harvest level.

Purpose: The objective of this study was to determine physical and/or microbial factors affecting the survival of Lm in different soil types.

Methods: The survival of two Lm strains in three soils under different management practice, (i.e., conventional agriculture (F1), fallowed for five years (F2), and uncultivated grassland (F3)), was monitored through daily spread plating on PALCAM agar. Soils were sampled from the soil surface (0-15 cm depth) and analyzed for a suite of physical and chemical properties. Microbial biodiversity analysis was conducted using high throughput sequencing (HTS) of 16s rDNA extracted from each soil sample.

Results: Area under the curve analysis revealed greater survival capacity for both Lm strains in F3 compared to F1 and F2 (Fisher's LSD, $P < 0.05$). Moisture content was found to be significantly different (Fisher's LSD, $P = 0.001$) between all three soils at 7.2%, 8.9%, and 10.2% for F1, F2, and F3, respectively, and F3 was significantly higher (Fisher's LSD, $P < 0.001$) in nitrogen at 25.3% compared to 13.5% and 21.2% for F1 and F2, respectively. Based on HTS data analysis, total reads were found to be higher in F1 compared to both F2 and F3 (Fisher's LSD, $P = 0.021$); however, at the phylum level, F3 was found to have a higher proportion of Proteobacteria (28.7%; Fisher's LSD, $P = 0.003$) and a lower proportion of Acidobacteria (8.7%; Fisher's LSD, $P< 0.001$) compared to both F1 (24.3% and 21.9%, respectively) and F2 (24.2% and 19.9%, respectively).

Significance: These results add to the current understanding of factors that may affect the risk of Lm occurrence and survival in soil environments.

T4-07 River Water as a Reservoir for *Salmonella enterica* on the Maryland Eastern Shore (Delmarva)

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❖ ***Developing Scientist Competitor***

Introduction: Foodborne illness outbreaks of *Salmonella enterica* have been associated with fresh produce cultivated on the Delmarva Peninsula. Epidemiological investigations linked a 2002 and a 2005 outbreak to tomatoes grown on the eastern shore of Virginia, with recovery of the outbreak strain from irrigation pond water on the implicated farm. The source of a 2014 *Salmonella* Newport outbreak in Maryland, associated with cucumber consumption, remains unknown. These recurrent outbreaks suggest a persistent environmental reservoir increasing the likelihood of contamination of fields in this region. We currently do not have sufficient environmental data to completely understand the ecology of *S. enterica* on the Delmarva Peninsula.

Purpose: The purpose of this study was to evaluate the prevalence of *S. enterica* in surface water from rivers on the eastern shore of Maryland (Delmarva).

Methods: Water samples were collected from a total of 23 sites along four major rivers (Choptank, Nanticoke, Wicomico, Pocomoke) on the eastern shore of Maryland during the summer and fall of 2015, to determine the presence of *S. enterica* in surface waters. At each sampling location, a 10 L volume of water was filtered through a Modified Moore Swab cartridge. *Salmonellae* were isolated by pre-enrichment in lactose broth, enrichment in tetrathionate broth, and selective growth on R&F *Salmonella* chromogenic plating medium. Presumptive *S. enterica* isolates were confirmed by PCR amplification targeting the invasion A (*invA*) and invasion gene regulator (*hilA*) genes.

Results: A total of 14 out of 23 (61%) samples were positive for *S. enterica* (1/6 Choptank, 5/5 Nanticoke, 5/6 Pocomoke, 3/5 Wicomico). From these samples, 110 presumptive *Salmonella* isolates were recovered (6 Choptank, 40 Nanticoke, 43 Pocomoke, 21 Wicomico). All isolates were PCR-positive for both the *invA* and *hilA* genes.

Significance: This study confirms that surface river water on the eastern shore of Maryland serves as an environmental reservoir for *S. enterica.*

T4-08 Associations of GI Microflora with *Campylobacter* Status in Commercial Broiler Chickens

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Introduction: Next-generation DNA sequencing is a powerful tool for food safety. One potential use for this technology is to compare the microflora of food animals hosting foodborne pathogens to pathogen-free animals to better understand the ecology of foodborne pathogens to design possible intervention strategies.

Purpose: The purposes of this study were to: 1) characterize the cecal microflora of commercial broilers (N=96) collected at harvest over the course of approximately one year (April 2013-May 2014), 2) determine the incidence of *Campylobacter* in these same samples, and 3) identify any taxa differentially represented in *Campylobacter*-positive versus negative birds.

Methods: Intact ceca were collected from whole GI tracts removed from commercial broilers at processing. Cecal contents were used for selective cultivation of *Campylobacter* and for characterization of the microflora using barcoded sequencing of 16S rRNA genes on the Illumina MiSeq platform.

Results: The composition of the cecal microflora was stable across seasons. The most abundant genera included *Bacteroides*, *Janthinobacterium* (phylum *Proteobacteria*), and *Clostridiales* such as *Butyricicoccus* and *Flavonifractor*. *Campylobacter* was detected in all sampling months with an average of 53% of samples positive. Comparisons of the microflora between *Campylobacter*-positive and negative samples showed two genera were significantly over-represented in *Campylobacter*-positive samples. These two genera, *Alistipes* and *Rikenella*, have been previously shown to be associated with intestinal inflammation.

Significance: To our knowledge, this study represents the first comparison of the microflora associated with *Campylobacter* status using next-generation DNA sequencing methods. As illustrated by the several genera identified here, this approach may be useful in identifying potential indicator taxa and may provide insights into the pathogenesis and ecological niche of *Campylobacter* as a foodborne pathogen.

T4-09 Survey of Foodborne Viruses in Australian Oysters at Production

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Introduction: Human enteric viruses are frequently associated with shellfish related foodborne outbreaks internationally. It has been suggested that acceptable virus limits be established for this high risk food group.

Purpose: To undertake a national prevalence survey for norovirus (NoV) and hepatitis A virus (HAV) in market-ready Australian grown oysters.

Methods: Sampling of oysters was done during 2014-15 in two rounds to capture "peak" and "off-peak" times for NoV circulating within the community. All commercial Australian production areas were included in the survey. A sample size of 150 per round was calculated based on the probability of 0.95 of detecting at least one positive if ≥2% of the samples were contaminated. Sample number allocation to production area was determined by its proportional contribution to national oyster production. Samples were only collected from growing areas in the *Open Status* for harvest, as defined by the Australian Shellfish Quality Assurance Program's Operations Manual. NoV GI, NoV GII and HAV were determined by quantitative RT-PCR (ISO/TS 15216-1).

Results: A total of 33 oyster production regions in New South Wales, South Australia, Tasmania and Queensland were involved in the survey. One hundred forty-nine and 148 samples were collected during round one and two of sampling, respectively. No NoV or HAV was detected in oysters collected in either sampling round. For each of the two sampling rounds this translated into an estimated prevalence for NoV and HAV in Australian oysters of <2% with a 95% confidence interval.

Significance: Results of this survey show that the Australian oyster industry is producing a safe product with respect to viral contamination. The low estimated prevalence of foodborne viruses in oysters was supported by epidemiological evidence, with no oyster-related foodborne illness reported during the survey period. There is no evidence that end product viral limits are necessary in the Australian context. Any end product viral limits should be applied on a risk assessment basis.

T4-10 Method Development for Detection of Human Norovirus in Produce Samples during an Outbreak Investigation

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Introduction: Noroviruses are the leading causes of outbreaks as well as sporadic cases of acute gastroenteritis worldwide. Although epidemiological evidence has indicated that norovirus is responsible for the majority of produce related outbreaks in both Europe and the United States there are still few occasions where it has been successfully detected from foods associated in outbreaks.

Purpose: Fruit salad was implicated in a recent norovirus outbreak in Wisconsin. An experimental investigation was undertaken to develop a method and test the implicated food vehicle.

Methods: Fifty grams of fruit salad (watermelon, cantaloupe, pineapple, honeydew and grapes) were artificially inoculated with norovirus. The protocol involved eluting the virus with a 0.1 M Tris-HCl, 0.05 M glycine, 1% beef extract, pH 9.2 (TGBE) buffer containing 2% Polyvinyl Pyrrolidone (PVP) and pectinase. After a brief spin, the eluate containing the virus particles was concentrated by pelleting virus via ultracentrifugation. Virus RNA was subsequently isolated from the pellet using a commercial kit, and viral genome detection accomplished using real-time RT-PCR.

Results: When fruit salad was artificially seeded, norovirus could be consistently detected at a level of at least 1,000 RNA copies per 50 g of sample. When the same experimental method was used to process samples from the outbreak, they all tested positive and more specifically contamination ranged from 10-1,000 RNA copies/sample.

Significance: Developing a robust method to detect norovirus in food samples associated with foodborne illness allows for a confirmatory tool for validation and application in response to outbreak management.

T4-11 Aptamer Binding Using Enzyme-linked Aptamer Sorbent Assay (ELASA) against Human Norovirus Virus-like Particles and Positive Stool Samples

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Introduction: The lack of rapid and sensitive assays to detect human norovirus contamination in food and environmental samples is one factor limiting the ability to control transmission. Nucleic acid aptamers are emerging ligands for use in pathogen capture and detection because of their high target binding affinity, ease of synthesis and functionalization, and stability.

Purpose: To evaluate the binding affinity and specificity of ssDNA aptamers previously selected against human norovirus GI virus-like particles (VLPs).

Methods: Six aptamer candidates (AP1-AP6) obtained after four rounds of a novel selection process were screened. Aptamer binding affinity was evaluated using an Enzyme-Linked Aptamer Sorbent Assay (ELASA) performed on a panel of human norovirus VLPs. Briefly, VLPs were bound to microtiter wells, blocked, and incubated with biotinylated aptamer. Plates were developed by the sequential addition of streptavidin peroxidase followed by a TMB 2-component microwell peroxidase substrate system. Absorbance was recorded at 450 nm and binding affinity was expressed as ratio of the absorbance of test sample to that of the negative control. Similar assays were done using 20% stool suspensions obtained from infected individuals.

Results: Of the six aptamers tested, AP4 displayed the greatest binding affinities for both GI and GII human norovirus VLPs. Average ratios for GI VLPS were 6.4±0.4 (GI.1), 5.2±0.6 (GI.6), 5.5±0.4 (GI.7), 5.0±0.4 (GI.8); and for GII VLPs were 4.0±0.4 (GII.1), 7.8±0.7 (GII.2 Snow Mountain), 6.0±0.3 (GII.4 Grimsby), 6.1±0.5 (GII.4 Houston), 6.6±0.7 (GII.12), 5.3±0.2 (GII.17). Difference in binding affinity were statistically significant by VLP type ($P<0.05$). Aptamers affinity for virus in diluted human fecal samples was dampened [GI.I (3.8±0.1), GI.6 (1.5±0.1), and GII.4 Sydney (1.1±0.03)], most likely due to matrix-associated inhibition.

Significance: The aptamers screened had broad reactivity and high binding affinity for a variety of human norovirus VLPs and are promising ligands for use in future capture and detection assays.

T4-12 Improvement of Virus Extraction from Soft Fruit by Implementing a PCR Inhibitor Removal Kit

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Introduction: Soft fruit has been associated with food-borne outbreaks. A horizontal method for the detection of norovirus (NoV) GI and GII and hepatitis A virus (HAV) in food using one-step RT real-time PCR has been published as ISO15216. Inhibitory substances from fruit often require the analyses of both undiluted and 10-1 diluted RNA.

Purpose: To examine whether the OneStep PCR Inhibitor Removal Kit (Zymo Research) can reduce RT-PCR inhibition levels in soft fruit samples.

Methods: Samples were analyzed according to ISO15216-2 with or without application of the kit just prior to RT real-time PCR. Inhibition was defined as amplification of $2x10^2$ DNA equivalents of in vitro transcribed HAV ssRNA external amplification control (EAC) with more than 2 Ct in undiluted sample RNA than in water.

Results: Selected RNAs derived from frozen soft fruit, previously demonstrated to contain inhibitory substances, were pooled per fruit type, resulting in 15 samples. Half of each pooled RNA sample was left untreated, the other half was cleaned-up. All treated samples had acceptable inhibition levels (< 2 Ct), whereas too much inhibition (>2 Ct) was shown for 11/15 (73%) of the untreated samples. Using artificially contaminated fresh soft fruit samples it was shown that the kit had no (substantial) negative effect on the detectability of NoV GI, NoV GII and HAV. Therefore, the kit was implemented in routine analyses. Without clean-up, 43% of the 145 samples collected in 2014 showed too much inhibition (>2 Ct) (17/47 strawberries; 4/15 blue berries, 14/34 blackberries and 27/49 raspberries). After implementation, all of the 115 soft fruit samples collected in 2015 (35 strawberries, 28 blue berries, 24 blackberries and 28 raspberries) had acceptable inhibition levels (<2 Ct).

Significance: With the clean-up method for RNA, all soft fruit samples had acceptable inhibition levels, which is an improvement of the method.

T5-01 BASELINE Software Tool for Calculation of Microbiological Criteria and Risk Management Metrics for Selected Foods and Hazards

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Introduction: The performance of risk-based metrics and the establishment of microbiological criteria could help to identify critical steps along the food chain that influence on the final risk associated to a specific pathogen.

Purpose: The aim of this paper was to illustrate how microbiological criteria (MC) for *Listeria monocytogenes* in cold-smoked salmon can be established based on risk-based metrics (Performance Objectives, PO). The example was achieved with the use of the Baseline software tool (www.baselineapp.com).

Methods: For simplification purposes, PO was set after product elaboration in the industry. The initial product contamination was 1.5 log CFU/g, storage at 4ºC during 4 days (96 h) and commercial salmon formulation was assumed: 2 ppm phenol and 3 mg/100g NaCl. The PO was set as no more than 5% of units in the lot would have a concentration above 3 log CFU/g. With this information an MC was established that just complies with the PO (95% CL).

Results: Growth increase of *L. monocytogenes* was 1.78 log CFU/g at the end of the storage period. The standard deviation of the product in such a way the PO will be complied [P (log CFU/g >3) < 5 % of the samples conforming the lot] was 0.74 log CFU/g. The MC is a practical tool to verify whether individual batches/lots meet the PO by taking samples from such batches/lots for microbiological analysis. Considering a microbiological limit of 2 log CFU/g, MC to allow lot rejection at 95% CL was derived through the Baseline software tool as n=7; c=0; m=2 log CFU/g.

Significance: The use of the Baseline software offers a flexible graphical user interface where models can be incorporated and/or evaluated as well as new sampling plans. This information may be used to evaluate the overall effectiveness of applied interventions by risk managers or food operators.

T5-02 Estimating Exposure in Quantitative Microbial Risk Assessment Models Using Dietary Recall Data

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Introduction: Quantitative microbial risk assessment (QMRA) models require information on how often a food is consumed and the amount consumed to characterize the risk from exposure in food. In the United States, the National Health and Nutrition Examination Survey (NHANES) provides comprehensive consumption data, but its use in QMRA is complicated. Data is obtained via two-day dietary recall over a two-year cycle from a subset of the population that oversamples specific sub-populations. Sample sizes from a single cycle are generally adequate, except for sub-groups and infrequently consumed foods. Therefore, multiple NHANES cycles are often used to improve precision in dietary exposure models but this assumes consistent consumption patterns over time.

Purpose: This study assessed the precision of the proportion of the population reporting consumption of a commodity and the average number of grams consumed per day, obtained with one versus two data cycles.

Methods: Dietary recall data from the 2009-2010 and 2011-2012 NHANES cycles were used to calculate, for individual and combined cycles, point estimates and 95% confidence intervals (CI) for 1) proportion of participants reporting consumption of a food commodity on either day, and 2) average grams consumed per day for days the commodity was consumed. Precisions of the estimates were compared to evaluate potential bias and variability.

Results: Minimal gains in precision were seen using two versus one cycle of NHANES data. For foods consumed by 0.1% of the population, the 95% CI were (0.4-0.19) and (0.06-0.16) for one and two cycles, respectively. Similarly, the estimated grams consumed per day for baked potatoes, consumed by 5% of the population, was 144.2 g/day (95% CI: 137.4-151.0) for one cycle and 147.0 g/day (95% CI: 141.5-152.5) for two cycles.

Significance: Improving the precision of dietary exposure estimates for QMRA is critical to providing actionable information to decision-makers.

T5-03 Comparison of Grouped Exposures for Estimation of Source Attribution of *Salmonella* Serotype Enteritidis Illness, Foodborne Diseases Active Surveillance Network

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Introduction: Estimating the proportion of sporadic (non-outbreak) *Salmonella* serotype Enteritidis (SE) illnesses that can be attributed to food sources and non-food routes of exposures is important for prioritizing and planning food safety policies. Current estimates of the major sources of SE illness are lacking.

Purpose: We proposed an analytic approach to estimate attributable fractions of grouped exposures by comparing exposure profiles of patients with SE infection to those infected with other *Salmonella* serotypes.

Methods: Patients living in FoodNet sites during January 2014 – October 2015 were asked about 48 food and environmental exposures. Missing exposures were imputed based on multiple imputation scheme. Related exposures were grouped into causal pathways for analysis by random forest model. Counterfactual simulation was conducted in which exposed statuses were changed into non-exposed ones and the influence of such changes on reduction of predicted illness was estimated.

Results: We examined data on 1,604 patients with SE infection and 5,667 infected with other serotype. The median proportion of missing information for the 48 exposures was 4% (range, 1% to 18%). The exposure frequencies of the imputed data were similar to those of the observed data. Analyses in which each of the three chicken exposures (consuming any chicken, chicken outside home, and consuming ground chicken) were removed from the model resulted in reductions of SE illnesses by 4%, 2% and 0%, respectively. However, removal of all three chicken exposures reduced SE illnesses by 14%, indicating interactions between nested exposures.

Significance: Our results suggest that case-case comparisons in a random forest model might provide estimates of the proportion of illness attributable to related exposures. Grouping all exposures related to a particular food category can provide important information for evaluating the impact of food safety policies and interventions that target that food.

T5-04 Using Genome-scale Metabolic Modeling to Compare Strains of the Foodborne Pathogen *Listeria monocytogenes*

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Introduction: *Listeria monocytogenes* is a microorganism of great concern for the food industry. Using a combination of computational techniques and laboratory methods, genome-scale metabolic models (GEMs) can be created, validated, and used to simulate metabolic capabilities of microbes of interest—including *L. monocytogenes.*

Purpose: The objective of this study was to generate GEMs for six different strains of *L. monocytogenes*, and to compare nutrient utilization predictions of these models to in vitro results.

Methods: Genomes for the six different strains of *L. monocytogenes* were taken from the NCBI database and uploaded to KBase—a semi-automated program used to generate the GEMs. These models were then used to generate nutrient utilization predictions for sources of carbon, nitrogen, phosphorus, and sulfur using General Algebraic Modeling System (GAMS) software. In silico predictions were then compared to in vitro experiments performed using Biolog phenotypic microarray plates. Following this comparison, GEMs were manually curated to increase agreement between in silico predictions and in vitro results.

Results: A total of 58 of the 95 carbon sources tested in vitro were present in the models, and; therefore, these were the compounds from which comparisons could be drawn. Of these 58 compounds, agreement between in silico predictions and in vitro results ranged from 79.3% to 89.7% between strains. For nitrogen, 62 of the 95 compounds were present, and agreement ranged from 59.7% to 66.1%. For phosphorus and sulfur, 33 of the 94 compounds were comparable, and agreement ranged from 36.4% to 45.5%.

Significance: These findings are significant because they show that these GEMs for *L. monocytogenes* are comparable in agreement between in silico predictions and in vitro results to published models of other organisms. Therefore, as with the other models—namely those for *Escherichia coli*, *Staphylococcus aureus*, *Vibrio vulnificus*, and *Salmonella* spp.—they can be used to determine new methods of growth control and disease treatment.

T5-05 The Importance of Data in *Salmonella* Risk Mitigation: Development of a Cloud-based Technical Platform for Food Safety Management in Poultry Production

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Introduction: Foodborne disease outbreaks represent an ever-present risk to human health and the poultry industry, with notable *Salmonella* outbreaks occurring in recent years. Outbreaks result in adverse health effects to the consumer, as well as negative brand impact and financial losses to companies. Following a multistate outbreak of *Salmonella* Heidelberg, a poultry producer worked with a biotechnology firm to collect, integrate, and analyze data across its operations.

Purpose: Develop a cloud-based technical platform for producers to collect, integrate, and analyze food safety and operational data, providing early indicators and predictive analytics associated with food safety pathogen emergence in the supply chain.

Methods: A technical platform is being developed to integrate, visualize, and analyze the company's national-level pathogen testing data; collection was for *Salmonella* in 2014–2015.

Results: Data was initially identified for 12,971 flocks, with the ability to trace production from breeder to broiler stage for only 17% of flocks. Less than 2% of flocks with *Salmonella* tests were traceable to *Salmonella* rehang tests in those same flocks at processing. Further investigation increased traceability to 22,471 flocks, with 100% flock traceability from pullet through processing for company-owned flocks. Linked flock and *Salmonella* test traceability from broiler to rehang stages improved to 10% of flocks. Food safety performance could now be examined over time at the individual flock level, to include: ability to trace flocks with increased positive *Salmonella* tests throughout the production chain; identification and correction of sampling bias at processing; and validation of a new sampling technique with better performance than the standard USDA carcass postchill test, resulting in an estimated savings of $42,893 per plant over an 18-month period.

Significance: The platform aims to provide operational insights at the flock level, which allows food safety managers to make evidence-based decisions to establish food safety priorities and evaluate interventions.

T5-06 Meta-analysis on the Effect of Interventions Used in Cattle Processing Plants to Reduce *Escherichia* coli Contamination

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Introduction: Cattle coming from feed lots to slaughter often harbor pathogenic bacteria that can contaminate final meat products. As a result, reducing contamination during processing is a main priority. Unfortunately plant managers face difficulty when trying to determine optimal intervention strategies from published literature. Intervention literature results and methods vary significantly, making it difficult to implement interventions with any degree of certainty in their effectiveness.

Purpose: To create a more robust understanding of plant intervention effectiveness by performing a formal meta-analysis on plant intervention literature. To assess factors involved in plant intervention effectiveness by explaining heterogeneity.

Methods: Available literature was gathered on popular intervention methods. Intervention effectiveness, measured as raw log reduction, was modelled using study characteristics, such as temperature of application, intervention type, starting microbial concentration, etc. Least-squares means were calculated for intervention effectiveness separately on hide and on carcass surfaces. Heterogeneity (I^2) was assessed and factors influencing intervention effectiveness were identified.

Results: Least-squares mean reductions for acetic acid, lactic acid, steam vacuum, and water wash on post de-hided surfaces (n=223) were 1.58 [95% CI: 0.76 - 2.40], 2.20 [1.46 - 2.94], 2.91 [2.19 - 3.62], and 1.85 [1.14 - 2.55] log CFU/cm^2, respectively. Least-squares means for acetic acid, lactic acid, sodium hydroxide, and water wash on hide surfaces (n=47) were 2.14 [1.42 - 2.87], 3.05 [2.32 - 3.77], 3.84 [2.94 - 4.73], and 0.11 [-0.79 - 1.01] log

Technical

CFU/cm^2, respectively. Heterogeneity, measured as I^2, was 94% and 92% for the carcass and hide models, respectively. Meta-regressions showed that temperature, pressure, pathogen starting concentration, sample method, surface type, and extra water wash were statistically significant covariates (P<0.10).

Significance: The results allow food safety specialists and risk assessors to evaluate plant intervention effectiveness, variation, and factors more adequately. Development of more robust decontamination procedures is possible, further reducing contamination and illness.

T5-07 Quantifying the Risk of Human *Toxoplasma gondii* Infection through Consumption of Domestically-produced Lamb in the United States

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Introduction: *Toxoplasma gondii* is a widely prevalent protozoan parasite that is able to infect humans and animals worldwide. Human toxoplasmosis is responsible for considerable morbidity and mortality in the United States. Meat products have been identified as an important source of *T. gondii* infections in humans. Lamb, defined as a sheep less than one year old and without permanent teeth, is one of the meat animals that is frequently infected with *T. gondii.*

Purpose: The goal of this study was to develop a farm-to-table quantitative microbial risk assessment (QMRA) model to predict the public health burden in the United States associated with consumption of U.S. domestically-produced lamb.

Methods: *T. gondii* prevalence in market lambs was pooled from the 2011 National Animal Health monitoring system (NAHMS) survey, and the concentration of the infectious life stage (bradyzoites) was calculated. A log-linear regression and an exponential dose-response model was used to model the reduction of *T. gondii* during home cooking and to predict the probability of infection, respectively.

Results: The mean probability of infection per serving of lamb was estimated to be 1.5 cases per 100,000 servings, corresponding to approximately 6,300 new infections per year in the U.S. population. Based on the sensitivity analysis, cooking was identified as the most effective method to influence human health risk.

Significance: This study provides a QMRA framework for *T. gondii* infection through consumption of lamb. The infection risk and public health burden associated with lamb consumption were quantified, which could help in managing *T. gondii* infection risk.

T5-08 Neural Network Models for Growth of *Salmonella* Serotypes in Ground Chicken Thigh Meat Subjected to Temperature Abuse during Refrigerated Storage

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Introduction: Predictive microbiology models are valuable tools for helping assess the risk of foodborne illness. However, different versions of the model may be needed to meet the divergent needs of the food industry and risk assessors.

Purpose: To develop multiple versions of a neural network model for growth of *Salmonella* serotypes in ground chicken thigh meat for the purpose of providing the food industry and risk assessors with models that meet their needs for improving the safety of chicken.

Methods: An automated miniature most probable number (MPN) method was used to enumerate *Salmonella* serotypes (n = 8) in ground chicken thigh meat portions (0.75 cm^3) during cold storage at 16°C for 0 to 8 days. A multiple-layer feedforward neural network model was developed using Excel and NeuralTools. Two additional versions of the Excel model were developed: a version for the food industry that does not require NeuralTools to run and a stochastic version for risk assessors that requires NeuralTools and @Risk to run. Performance of the model was evaluated using the acceptable prediction zone method.

Results: Growth of *Salmonella* was affected (P < 0.05) by serotype at 4, 6, and 8 days of storage but not at 0, 1, or 2 days of storage at 16°C. Maximum log MPN per portion ranged from 6.12 ± 0.47 (mean ± SD) for serotype 8,20:-:z_6 to 6.84 ± 0.23 for serotype Thompson. The proportion of residuals in an acceptable prediction zone (pAPZ) from -1 log (fail-safe) to 0.5 log (fail-dangerous) was 0.948 for training data (n = 192) and 0.988 for testing data (n = 84). A pAPZ ≥ 0.7 indicates that the model provided predictions with acceptable bias and accuracy. Thus, the model was successfully validated.

Significance: The new models will allow the food industry and risk assessors to better assess the risk of salmonellosis from chicken subjected to temperature abuse during refrigerated storage.

T5-09 Evaluating the Performance of a New Model for Predicting the Growth of *Clostridium perfringens* in Cooked, Uncured Meat and Poultry Products under Isothermal, Heating, and Dynamically Cooling Conditions

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Introduction: *Clostridium perfringens* Type A is a significant public health threat. It may germinate, outgrow, and multiply during cooling of cooked meats. Computer simulation may be used to evaluate the growth of *C. perfringens* in cooked meats during cooling.

Purpose: The objective of this study is to evaluate the performance of a new *C. perfringens* growth model in IPMP Dynamic Prediction for predicting its growth under dynamic cooling, isothermal, and dynamic heating temperature profiles.

Methods: Computer simulation is used to predict the growth of *C. perfringens* under different temperature profiles. The residual errors of predictions (observation-prediction) are analyzed, and the root-mean-square errors (RMSE) calculated.

Results: For isothermal and heating profiles, each data point in growth curves is compared. The mean residual errors of predictions (MRE) range from -0.40 to 0.02 log CFU/g, with a RMSE of ~0.6 log CFU/g. For cooling, the end-point predictions are conservative in nature, with an MRE of -1.16 log CFU/g for single rate cooling and -0.66 log CFU/g for dual rate cooling. The RMSE is between 0.6 and 0.7 log CFU/g. Compared with other models reported in Mohr and others (2015), this model makes comparable or mostly better accurate and fail-safe predictions. For cooling, the percentage for accurate and fail-safe predictions is between 97.6% and 100%. Under criterion 1, the percentage of accurate predictions is 47.5% for single rate and 66.7% for dual rate cooling, while the fail-dangerous predictions are between 0 and 2.4%.

Significance: This study demonstrates that IPMP Dynamic Prediction can be used by food processors and regulatory agencies as a tool to evaluate the safety of cooked or heat-treated uncured meat and poultry products exposed to cooling deviations or to develop customized cooling schedules. This study also demonstrates the need for more accurate data collection during cooling.

T5-10 Risk Factors for Prevalence and Concentration of Indicator Microorganisms on Fresh Tomatoes in the Postharvest Supply Chain

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Introduction: In risk assessment models, "prevalence" and "concentration" describe microbial populations in product units, capturing rare but significant occurrence of contamination. While estimates for human pathogens are difficult to obtain experimentally or by regular surveillance, indicator microorganisms can demonstrate similar potential behavior if pathogens are introduced postharvest.

Purpose: Here, the objective was to use microbial indicators of quality and safety on fresh tomatoes to determine and estimate the significant factors of the postharvest supply chain that influence these characteristics, prevalence and concentration.

Methods: The factors included were location in the supply chain, harvest date, and days in transit. Microbial count data (log CFU/tomato) for aerobic mesophiles (APC), total coliforms (TC) and yeasts/molds (YM) on the surface of Roma tomatoes (n=475) sampled within lots moving through a supply chain were used in mixed linear models (PROC MIXED, SAS 9.3) to determine significant factors for concentration. Based on a detection limit of 0.3 log CFU/tomato, count data were converted into a binomial variable and modeled using logistic regression (PROC GENMOD, SAS 9.3) to estimate the prevalence of tomatoes with detectable indicators.

Results: Location explained prevalence changes in TC (p=.0009) and YM (p<.0001), while days-in-transit best explained concentration dynamics in all populations (p<0.001), with each additional day contributing 0.5 log on average. Used together, these models quantified the dynamics observed (% prevalence, LS mean±s.e.). For example, at harvest TC had low prevalence in sampled tomatoes (13%), but high concentrations (2.7±0.5 log). After packing, TC prevalence (53%) and concentration (3.1±0.4 log) increased, while at distribution both decreased (30%, 0.6±0.2 log). At supermarkets, prevalence increased (55%) while concentration was variable (0.3-4.2 log/tomato).

Significance: Locations with increased prevalence and variability were packinghouse and retail and the difference in concentration between a six- and ten-day supply chain was 2 log CFU/tomato. These results can be used in future risk assessment models.

T5-11 Development of a System Model to Predict the Impact of Pre-harvest Contamination Sources on a Possible Leafy Greens-related *E. coli* O157:H7 Outbreak

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Introduction: Consumption of leafy greens contaminated with pathogens remains a major cause of foodborne illnesses in the United States. For many outbreaks associated with pre-harvest contamination of leafy greens, the source of pathogens origination is unclear because of challenges involved in conducting traceback investigations. Salinas Valley in California is a major producer of leafy greens, but at the same time, has been linked to more than ten *Escherichia coli* O157:H7 outbreaks associated with leafy greens produced in this region since 1999.

Purpose: The objective of this study was to develop a pre-harvest system model to understand the ecology of *E. coli* O157:H7 in leafy greens production.

Methods: A dynamic system model consisting of subsystems (soil, irrigation, livestock operations, rainfall, and wildlife) simulating a farm in Salinas Valley was developed. This model assumed two crops of lettuce in a year. The model was simulated assuming the events of plantation, irrigation, harvesting, tillage, contamination of soil and plants, and survival of *E. coli* O157:H7.

Results: The concentrations of *E. coli* O157:H7 in the crops harvested in different months as predicted by the baseline model for conventional fields estimated that 11 out of 221 first crops harvested in July will have at least one plant with more than 1 CFU of *E. coli* O157:H7. The concentrations were higher in the second crops than the first crops, with the probability of having at least one plant with more than 1 CFU of *E. coli* O157:H7 in a crop predicted as 21/253, 4/333, 11/307, and 6/105 in August, September, October, and November, respectively. For organic fields, the probabilities of having at least one plant with more than 1 CFU of *E. coli* O157:H7 in a crop were predicted to be higher than those for the conventional fields.

Significance: These results are in close agreement with the known outbreaks and could be useful in developing metrics to mitigate the risks of leafy greens associated outbreaks.

T5-12 Evaluating Intervention Strategies to Reduce the Burden of Foodborne Disease Caused by Human Norovirus: A Risk-Based Approach Using a Long-term Care Facility as Proof of Concept

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Introduction: Human norovirus causes 19-21 million cases of illness in the U.S annually, about 5 million of which are transmitted by foods. Long-term care facilities (LTCFs) are among the most common settings for norovirus outbreaks (78% of outbreaks reported in the U.S., 2009-2013), and facility food services are recognized as a significant contributor to disease burden. Development of an effective food safety plan that addresses key risk factors is essential.

Purpose: The primary purposes of this work were to (1) develop a quantitative predictive risk assessment model that simulates norovirus disease dynamics, and (2) provide a comparative risk-cost framework to evaluate the efficacy and cost-effectiveness of potential intervention strategies.

Methods: We developed an agent-based model, NorOPTIMAL, that simulates norovirus transmission dynamics and disease in a LTCF. NorOPTIMAL represents interactions among agents (e.g., health care workers), and between agents and the microenvironment (e.g., surfaces, foods). Model parameters were derived through an exhaustive literature review, research conducted by the NoroCORE food safety initiative, and expert judgment. To demonstrate the utility of NorOPTIMAL to inform development of a risk-based food safety plan, a hypothetical case study was created that compared different intervention strategies for reducing foodborne contamination and spread of norovirus.

Results: The notional results from the case study suggest that a multi-option intervention strategy is most cost-effective in reducing disease burden including, for example, adoption of best sanitation practices for areas impacted by a vomiting event, rapid response training to limit residents' exposure to aerosolized virus particles, and increasing compliance with personal hygiene requirements in the kitchen area.

Significance: This work provides: (1) a mathematical framework for simulating transmission dynamics of norovirus; (2) a teaching tool to learn risk assessment basics in a hands-on simulation environment; and (3) a practical approach to compare proposed intervention strategies and inform the development of an effective food safety plan.

T6-01 Susceptibility of Aged C57BL/6 Mice to *Listeria monocytogenes*: A Potential Surrogate Model for Human Foodborne Listeriosis in the Aging Populations

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Introduction: Foodborne *Listeria monocytogenes* (LM) is a leading cause of serious illness and death in the US. The case-fatality rate of invasive LM infection in the elderly population is >50%.

Purpose: The aim of this study is to establish a murine model of oral LM infection to evaluate dose-response and immune response during listeriosis between the aged population and the younger populations.

Methods: Young-adult (3 months age), 14-months old, and 22-months old C57BL/ 6 mice were orally gavaged for two consecutive days with doses ranging from 2 x 10^6 to 2 x 10^9 CFU/mouse with a murinized LM strain (Lmo-*InlA*^{m}) and monitored for body-weight changes and survivability. Tissues were collected and assayed for bacterial burden, histology. Also, in vitro stimulation (LM Ag/lysate with/wo anti-CD3/CD28) assays were performed using splenocytes isolated from uninfected or LM-infected young and old mice to compare immune biomarkers and proliferative responses. Supernatants were assayed for cytokine secretion (IL-2, IL-1β, TNF-α, IL-17, IL-10 and IFN-γ) using BioPlex assays.

Results: Compared to adult mice, the mortality rate for the older (14-months) C57BL/6 mice was relatively higher (7% vs 46%). Older mice lost significantly (P=0.005, ANOVA) more body-weight in a dose-dependent manner and had a higher bacterial burden in liver (P<0.05) and spleen (P=0.05) when compared to adult infected mice. LM-lysate treated splenocytes from older mice produced significantly (P<0.05) higher levels of the anti-inflammatory cytokines (e.g., IL-10) and higher levels of IL17, a pro-inflammatory cytokine, but produced less IFN-g. Splenocytes from the older mice proliferated significantly less and produced less IL-2 as compared to young mice.

Significance: These data suggest that older C57BL/6 mice are more susceptible to LM infection due to an imbalance of Th cell responses with disproportionate increase of anti-inflammatory responses and decreased proliferative capacity of immune cells.

T6-02 Metabolomic Analysis of Acid Stress Response in Shiga Toxin-producing *E.coli* O26:H11

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Introduction: Shiga toxin-producing non-O157 *E.coli* (STEC) strains such as *E.coli* O26 are responsible for a growing number of food-related illnesses around the world. Bacterial pathogens usually encounter pH levels around 3.6 in fruits and juices and pathogen outbreaks associated with such foods occur. A mechanistic understanding of acid stress in non-O157 STEC can help refine the "hurdles" used in the food industry.

Purpose: Metabolomic biomarkers accumulating in *E.coli* O26:H11 when exposed to acid (pH 3.6) conditions in strawberries was compared to a non-acidic (pH 7.5) control buffer.

Methods: Synthetic (pH 7.5) buffer and strawberry puree (pH 3.6) were used to identify the metabolites accumulating in a high titer preparation of *E.coli* O26:H11 cells during a 24-hour exposure to pH 3.6 in the strawberry puree compared to the control buffer. Culture methods and viability assays monitored the pathogen's survival while TEM was used to verify the structural integrity.

Results: Only a 1-log decline in the pathogen numbers occurred when exposed to the strawberry (pH 3.6) puree. Untargeted metabolomic analysis identified 293 metabolites out of which 155 were differentially (P < 0.01) expressed in the strawberry puree. Six different metabolic pathways were differentially triggered (P < 0.01) after 24 hours of exposure in the strawberry puree The pathways were D-Glutamine/D-glutamate, purine, pyrimidine, peptidoglycan biosynthesis, citric acid cycle, and Glyoxylate/dicarboxylate metabolism. Viability assays and TEM confirmed the structural integrity of the pH 3.6 exposed cells.

Significance: The results demonstrate that exposure of *E.coli* O26 to a strawberry (pH 3.6) matrix elicits a unique set of metabolic biomarkers. The metabolic pathways are preserving the structural integrity and viability when exposed to acid (pH 3.6) stress in the strawberry matrix.

T6-03 Quantifying the Effects of Acid (pH 3.6) Stress on Non-O157 Shiga Toxin-producing *Escherichia coli* Strains

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Introduction: In the US, non-O157 Shiga toxin-producing *Escherichia coli* (STEC) may account for up to 20%-50% of all STEC infections. Because of the high incidence rate and the relatively little that is known about non-O157 STEC, it is important to study and understand how these pathogens respond to acid stress encountered in acidic foods or used as "hurdles" in the food industry.

Purpose: The focus was to investigate how non-O157 STEC strains namely STEC O26, O45, O145, O111, O121, and O103 strains respond to a pH level of 3.6, normally encountered in berries such as strawberries.

Methods: A high titer (10^9 CFU) preparation of the different STEC strains was incubated in an acidic (pH 3.6) buffer solution and in a strawberry (pH 3.6) puree for 24 hours at room temperature. The pH 3.6 level was used to emulate acid stress routinely occurring in fresh berries. The number of survivors after 24 hours of acid stress exposure was then determined.

Results: The pH 3.6 acid buffer resulted in approximately 6 ± 1.1 log reductions of the STEC strains while the pH 3.6 strawberry puree only caused a 1.4 ± 0.67 log reduction of the STEC strains. STEC O45 and O145 strains were the most resistant to acid stress, while O26 and O121 strains were the most sensitive to acid stress.

Significance: The pathogens are significantly more resistant to pH 3.6 in a fruit matrix compared to a pH buffer. These results suggest that acid stress response in STEC strains is mechanistically different depending on the matrix in which the exposure occurs. The underlying metabolomic mechanisms need to be elucidated.

T6-04 Down-Regulation of Flagellin in CytR Mutant Leads to an Attenuation in Virulence of *Escherichia coli* O157:H7

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Introduction: *Escherichia coli* O157:H7 is a foodborne pathogen causing an estimated annual incidence of 73,000 cases of diarrheal illness in the United States. Cytidine repressor (CytR) plays a crucial role in transcription initiation from several operons in pathogens. However, little is known about its contribution to the virulence of *E. coli* O157:H7.

Purpose: To evaluate the possible molecular mechanism for CytR in pathogenesis of *E. coli* O157:H7.

Methods: The CytR insertion mutant strain (ΔCytR) was constructed by using TargeTron® Gene Knockout System. *E. coli* O157:H7 wild-type strain (WT, ATCC 43894) and ΔCytR strain were cultured for 16 h in THY medium at 37°C. The prominent differential expression protein between the two strains was analyzed by NanoLC-Nanospray QTOF MS-MS/MS. The transcription level of differential genes was analyzed by using quantitative RT-PCR (qRT-PCR), which was repeated three times. The virulence of the two strains was investigated via an oral infection mouse model with 3-5×10^{10} CFU.

Results: Compared with the WT strain, SDS-PAGE and NanoLC-Nanospray QTOF MS-MS/MS analyses indicated that flagellin was significantly decreased in ΔCytR strain (P<0.01). qRT-PCR analysis indicated that the transcription level of flagellin was dramatically inhibited in ΔCytR strain compared to WT strain (P<0.01). The survival rate in WT group and ΔCytR group was 60% and 100% (P<0.05), respectively.

Significance: The expression of flagellin, a principal component of bacterial flagella being responsible for adhesion and virulence of *E. coli*, was down-regulated in CytR-deficient strain, leading to the attenuation of virulence. Our result suggests that CytR or flagellin could serve as a novel drug target in controlling the pathogenesis of *E. coli* O157:H7. To identify small compounds as candidates for inhibition of *E. coli* O157:H7 attachment, a high-throughput screening assay is developing based on a chloramphenicol resistance gene under control of the CytR or flagellin promoter.

T6-05 Determination of the Chaperon Protein DnaK Production of the Big Six Non-O157:H7 Shiga Toxin-producing *E. coli* (STEC) under Heat- and Acid-shock by Competitive Enzyme-Linked Immunosorbent Assay (ELISA)

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Introduction: The emerging foodborne non-O157:H7 Shiga toxin producing *E. coli* (STEC) have been recently added to the zero tolerance policy by the USDA FSIS. Therefore, the characterization of their ability to adapt to stress encountered in the food environment that can be provided by the chaperon, heat shock protein, DnaK needs to be determined.

Purpose: The objective of this study is to compare the levels of DnaK protein under heat-shock and acid-shock conditions among different STEC strains.

Methods: Overnight cultures of six non-O157 STEC, one *E. coli* O157:H7 (EDL 933) and one non-pathogenic *E. coli* K12 were grown in tryptic soy broth (TSB). For the heat shock treatment, cells were held at 46°C for 25 min prior to protein extraction. For the acid shock treatment, cells were held in acidified TSB with acetic acid 0.50M (pH 6.5) for 1 h at 37°C. Cells were disrupted using a commercial enzyme cocktail followed by the addition of phenymethylsulfonyl fluoride to inhibit proteases and extract DnaK. A competitive ELISA was used to determine the intracellular DnaK concentration using absorbance at 450 nm. Each experiment was replicated thrice and data were statistically analyzed.

Results: Significant differences (P>0.05) in the intracellular concentration of DnaK were found between the tested strains exponentially grown at 37°C with values ranging from 1,821±120 to 6,204±423 molecules per cell. The values observed for heat shock and acid shock ranged from 6,867±340 to 18,182±460 and 5,032±475 to 15,919±549 molecules/cell, respectively.

Significance: The monitoring of the internal concentration of DnaK proteins shows that varying DnaK concentrations are detected during sub-lethal injury with either heat or acid stress. The elevated concentration of DnaK is associated with the ability of the cells to adapt to stress. Therefore heat shock proteins can potentially be used in addition to cell counts to determine the susceptibility of STEC to adapt to or survive different food processing conditions.

T6-06 Characterization of the Cytolethal Distending Toxin in Nontyphoidal *Salmonella* Serotypes Commonly Associated with Human Cases of Salmonellosis in the United States

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Introduction: Recently, multiple serotypes of nontyphoidal *Salmonella* (NTS) were found to encode an active cytolethal distending toxin (CDT), which causes DNA damage and a G2/M cell cycle arrest in eukaryotic cells. The implications of CDT in the context of NTS, with regards to CDT status among serotypes as well as potential differences in the outcome of infection with CDT positive and CDT negative strains, are poorly understood.

Purpose: The goals of this study were to (i) determine the distribution of CDT among NTS serotypes, and (ii) characterize differences in the outcome of infection with CDT positive and CDT negative strains using a cell culture model system.

Methods: A total of 866 strains belonging to 21 of the most frequently isolated NTS serotypes from cases of human salmonellosis were screened using PCR amplification of internal regions of *cdtB, pltA,* and *pltB*, encoding the CDT. Toxin activity was confirmed using flow cytometry to compare cell cycle progression of HeLa CCL-2 cells infected with CDT-positive and CDT-negative strains.

Results: Genes encoding a complete CDT were detected in 100% of strains (n=50 tested per serotype) for serotypes Javiana, Montevideo, and Oranienburg, and for 50% (4 out of 8) of strains for serotype Mississippi. HeLa cells infected with wild type strains of Javiana, Montevideo, and Mississippi exhibited a characteristic G2/M phase arrest, indicative of these strains producing an active CDT. By comparison, HeLa cells infected with either mutants lacking the gene encoding the active component of CDT, or CDT-negative strains, did not arrest in the G2/M phase.

Significance: These results suggest that infection with CDT-positive strains of NTS impart DNA damage and may result in a different illness compared to CDT-negative strains. Furthermore, these results are relevant for determining the public health impact of CDT-positive NTS infections.

T6-07 Influence of Ethanol Adaptation on *Salmonella enterica* Serovar Enteritidis Survival in Acidified Media and Selected Fruit Juices

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Introduction: *Salmonella enterica* serovar Enteritidis can encounter mild ethanol stress during its life cycle. Adaptation to the stress may later protect bacteria against heterogeneous stresses, thus counteracting the effectiveness of currently employed food control measures.

Purpose: This work was undertaken to evaluate the ability of *Salmonella* Enteritidis to develop acid resistance following ethanol adaptation.

Methods: *Salmonella* Enteritidis was subjected to ethanol adaptation in 5% ethanol for 1 hour. The tolerance of ethanol-adapted *Salmonella* Enteritidis to acetic, ascorbic, lactic, citric, malic and hydrochloric acids was assessed by the modulation of minimum inhibitory concentration and minimum bactericidal concentration values. The survival rates of non-adapted and ethanol-adapted *Salmonella* Enteritidis in refrigerated (4°C) and room temperature (25°C) stored apple juice and orange juice were also compared.

Results: Ethanol-adapted *Salmonella* Enteritidis only mounted cross-tolerance to one of the six acids tested, namely, malic acid. The survival of *Salmonella* Enteritidis in orange juice was not significantly (P>0.05) influenced by ethanol adaptation. However, an increased tolerance was noted with ethanol-adapted cells of *Salmonella* Enteritidis compared to non-adapted ones in apple juice stored at 25°C (P < 0.05), but not at 4°C.

Significance: These findings suggest the convenience to control the storage temperature as a strategy to prevent the development of cross-protection in acidic fruit juices. Furthermore, the combination of ethanol and malic acid applied sequentially should be avoided in food-related environments.

T6-08 Effects of Meat Juice on Biofilm Formation of *Salmonella* and *Campylobacter*

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Introduction: *Salmonella* and *Campylobacter* are both leading causes of foodborne illnesses worldwide. Raw meat is common food vehicle for *Salmonella* and *Campylobacter* that are prevalent in meat processing environment in the form of biofilms and further contribute to cross-contamination and food poisonings.

Purpose: This study applied meat (chicken and pork) juice as a minimal processed food model to investigate its effect on *Campylobacter* and *Salmonella* biofilm formation for a better understanding of biofilm formation in the real meat processing environment.

Methods: Meat juice was collected during raw meat freeze-thaw process and then sterilized by filtration. Bacterial biofilm formation level was determined using a live cell staining method, which is based upon the metabolic activity of bacterial cells. Confocal laser scanning microscope (CLSM) coupled with fluorescence cell stain was used to characterize the biofilm 3-D structure.

Results: In polystyrene 96-well plates, over 25% of chicken juice supplemented in Mueller Hinton broth (v/v) could significantly enhance the survival of *Campylobacter* in its biofilm (P<0.05). For *Salmonella*, Luria Bertani broth supplemented with 10% chicken juice (v/v) could lead to a significant increase compared to the control group (P<0.05). Similarly, meat juice could also support biofilm formation of *Campylobacter* and *Salmonella* in a microfluidic "lab-on-a-chip" platform, which was under a well-controlled flow condition. During the initial attachment stage of biofilm formation, more bacterial cells were present on the substrate conditioned with meat juice residues, compared to clean substrate materials. In CLSM images, the biofilms demonstrated different distribution patterns between laboratory media and meat juice.

Significance: These results validated the variation in *Campylobacter* and *Salmonella* biofilm formation between different conditions. In application, thorough cleaning of meat residues during meat production and handling are considered to be critical in reducing the load of *Campylobacter* and *Salmonella* in the environment and agri-food products.

T6-09 Transcriptome Analysis for Invasive *Staphylococcus aureus* Strains by Next Generation Sequencing

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❖ ***Developing Scientist Competitor***

Introduction: *Staphylococcus aureus* are known to cause foodborne illness by intoxication. However, *S. aureus* strains which can invade Caco-2 cell were recently identified. Hence, the pathway of *S. aureus* infection should be elucidated.

Purpose: The objective of this study was to determine the invasion pathway of *S. aureus* using next generation sequencing.

Methods: Among *S. aureus* strains, two strains were selected for invasion positive (*S. aureus* KACC10768) and invasion negative (*S. aureus* KACC10778) according to the results of previous studies. To construct cDNA libraries, 1 µg of total RNA was used. For RNA fragmentation, random hexamer primed reverse transcription and 100 nt paired-end sequencing by Illumina HiSeq2000. The libraries were quantified using qPCR and qualified using Agilent Technologies 2100 Bioanalyzer. To estimate expression levels, the RNA-Seq reads were mapped to the genome of *S. aureus* using TopHat. The reference genome sequence of *S. aureus* and annotation data were downloaded from the Pepper Genome Platform (PGP) ftp site. The transcript counts in isoform level were calculated, and the relative transcript abundances were measured in FPKM using Cufflinks. In addition, novel transcripts were found per sample. These results were obtained by Cufflinks Reference Annotation Based Transcript Assembly (RABT) method, allowing discovery of reference transcripts and novel transcripts. All data analysis and visualization of differentially expressed genes was conducted using *R*.

Results: Relative expression for attachment related genes was higher (P<0.05) in invasion positive strain than in invasion negative strain, and various regulator genes especially for translation were highly expressed in invasion positive strain. However, the relative expression levels for most stress response genes were not different between two strains.

Significance: This result indicates that invasive *S. aureus* has high relative expression level for attachment-related gene and regulator genes, especially for translation.

T6-10 Autoaggregation in *Cronobacter sakazakii* ATCC 29544 Is Mediated by *Flagella*

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Introduction: *Cronobacter sakazakii*, an opportunistic pathogen, causes nosocomial and food-borne infections in infants and the elderly. Currently, little is known about the mechanisms that *C. sakazakii* employs during gastrointestinal colonization and biofilm formation. One potential mechanism utilized by several human pathogens is autoaggregation, the clumping of taxonomically-related bacteria, which may promote formation of dense sessile colonies during the initial stages of colonization and biofilm formation.

Purpose: The purpose of this study was to identify the mechanism of autoaggregation in *C. sakazakii* ATCC 29544 (CS29544).

Methods: CS29544 was grown aerobically with agitation at 37°C. Stationary phase cells were stagnantly incubated at 25°C for 2 h to observe autoaggregation. Stable non-autoaggregative CS29544 were isolated following successive passages (6 total) of the uppermost bacterial cells. The resulting non-autoaggregators and CS29544 were sequenced using the Illumina platform to identify single nucleotide polymorphisms (SNPs). Candidate genes

(containing SNPs) were identified by BLAST and confirmed by Sanger sequencing. Candidate genes were disrupted in the CS29544 wild-type using the lambda Red recombinase system. Presence or absence of flagella was determined by either flagella stain or transmission electron microscopy.

Results: Initially, CS29544 was found to autoaggregate within 30 min; however, the growth media didn't fully clarify. Using selective enrichment, we isolated two independent non-autoaggregating variants of CS29544. Comparison of the variant genomes to the wild-type genome revealed unique SNPs in two flagella proteins; flhA and fliG. The disruption of flhA and fliG, along with fliC, in the wild-type resulted in non-autoaggregating CS29544 that did not possess extracellular flagella. Disruption of the motor genes, motAB, didn't affect autoaggregation. In conclusion, autoaggregation in CS29544 is mediated by the presence of structurally-intact flagella.

Significance: Understanding the mechanism of autoaggregation in *C. sakazakii* will provide novel insights into how *C. sakazakii* initiates formation of biofilms and colonization of the gastrointestinal tract.

T6-11 Mammalian Cell-Based In Vitro Pathogenicity Analysis of *Listeria monocytogenes* Biofilm-forming Cells

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Introduction: *Listeria monocytogenes* is a gram-positive facultative intracellular foodborne pathogen that causes listeriosis, and could be fatal to immunocompromised individuals such as the elderly, pregnant women, infants and the AIDS patients. Biofilm forming strategy facilitates the pathogen to survive and thrive on food-contact surface, which could be a significant threat to public health.

Purpose: This study compares the pathogenicity of biofilm-forming and planktonic *L. monocytogenes* cells as to their virulence genes expression, adhesion, invasion and cytotoxicity to mammalian cells.

Methods: Firstly, the biofilm-forming capability of over 100 *L. monocytogenes* strains, including the food- and clinical-isolated cultures, was screened using crystal violate staining method. Secondly, 6 strains representing high and medium biofilm-forming groups were selected for further in vitro pathogenicity study. Two types of human intestinal epithelial cell lines, Caco-2 and HCT-8, were used for in vitro adhesion and invasion experiment. A B cell hybridoma, Ped-2E9, was applied to test the cytotoxicity of *L. monocytogenes* using flow cytometry. Then, reverse transcriptional PCR (qRT-PCR) was used to test the expression of genes related to flagellum biosynthesis (*flaA*), pathogenesis (*lap* and *inlA*) and quorum sensing (*luxS*).

Results: In general, *L. monocytogenes* food isolates showed higher biofilm forming phenotype than the clinical isolates. The results of in vitro experiments using Caco-2, HCT-8 and Ped-2E9 cells suggest that biofilm-forming *L. monocytogenes* cells grown in minimal nutrient medium had significantly decreased adhesion, invasion and cytotoxicity capabilities compared to the planktonic cells grown in rich or poor nutrient media. Furthermore, qRT-PCR results indicated that *flaA* gene was over expressed in the biofilm-forming cells compared to the planktonic cells. While *luxS* and virulence genes (*lap* and *inlA*) were underexpressed during biofilm formation and overexpressed in planktonic cells.

Significance: These results reveal that the virulence properties of *L. monocytogenes* diminish when the pathogen switches from planktonic cells to biofilm-forming cells.

T6-12 Heat Resistance Markedly Varies between Different Strains of Human Norovirus

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Introduction: There are many physical and chemical methods that can be used for inactivation of human noroviruses. Many of these inactivate the viruses because of destruction of the capsid, which binds to host cells to initiate infection. Noroviruses display wide diversity in capsid amino acid sequence but the effect this diversity has on resistance to inactivation has not been investigated.

Purpose: Investigate the degree and mechanism of strain-specific variability in human norovirus heat resistance.

Methods: Virus-like particles (VLPs) of two different epidemic genotype GII.4 strains (Sydney, SYV; Houston, HOV) and one GII.2 strain (Snow Mountain, SMV) were used. The VLPs were subjected to different heat treatments (time-temperature combinations) and capsid functionality assessed using receptor and aptamer binding assays. The impact of heat on VLPs was also analyzed using dynamic light scattering (DLS) and transmission electron microscopy (TEM). Ligand docking and molecular dynamics (MD) simulations were done to predict capsid structural differences that mediate thermal resistance.

Results: SMV and SYV displayed much greater heat resistance than HOV. For example, treatment at 70°C for one minute resulted in loss of 47.6±5.3% of binding signal for HOV but had little impact on SYV (2.7±4.5% loss) or SMV (0.9±2.6% loss). At 65°C, 36.3±6.1% of receptor binding for SYV was lost after 25 minutes while HOV lost 35.5±9.2% after only 3 minutes. DLS and TEM data confirmed the ligand binding findings. Ligand docking simulations located aptamer binding in the same capsid region as histo-blood group antigen binding. MD simulations revealed that HOV had lower P-to-P-domain hydrogen bonds and more solvent accessible area compared to SYV and SMV.

Significance: Human norovirus displayed significant strain-to-strain variability in thermal resistance, a phenomenon likely associated with capsid structure. These differences have implications for food and environmental control measures, especially given the high degree of epidemic GII.4 strain capsid sequence variability.

T7-01 Antimicrobial Properties of a Multifunctional Carbohydrate Complex against Foodborne Pathogens

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Introduction: Antimicrobial compounds are making their way into food packaging materials in an effort to add an additional layer of protection against foodborne outbreaks from pathogenic bacterial contamination. Natural carbohydrates are attractive compounds as antimicrobial agents in food products due to their biocompatibility but they require an efficient immobilization onto package linings.

Purpose: The objective of this study was to identify and synthesize carbohydrate complexes that could be used as antimicrobial coatings on plastic packaging materials.

Methods: Carbohydrates were explored for antimicrobial properties. A carbohydrate complex on magnetic nanoparticle support (MNP-F#2) was synthesized, suspended in water, applied as droplets on plastic strips (F#2-strip), and heated for attachment. Three types of plastic packaging materials were employed for the antimicrobial experiments in 25 ml of milk samples (Vitamin D, 2%, and fat free). Phosphate buffer solution (PBS) was used as control matrix. Antimicrobial effects were monitored by bacterial growth and transmission electron microscopy (TEM).

Results: By itself, the carbohydrate complex did not attach to the plastic packaging. Successful attachment was achieved when it was immobilized on magnetic nanoparticles. The MNP-F#2 stayed attached on plastic strips even when exposed to water, milk or phosphate buffer solution over a longer period of time. The carbohydrate complex acted as both ligand and antimicrobial agent against *Salmonella* Enteritidis (Se), *E. coli* O157:H7 (Ec), *Bacillus cereus* (Bc), and *Listeria monocytogenes* (Lm). Ligand action was observed to be on the flagella for Se and on surface molecules for the other bacteria as evidenced through TEM images. Antimicrobial effects were observed to damage bacterial cell walls leading to cell lysis and growth inhibition.

Significance: We have successfully synthesized a very inexpensive antimicrobial carbohydrate complex that could be used to coat plastic packaging materials. The carbohydrate complex may be useful for other antimicrobial applications.

T7-02 Natural Antimicrobial for Methicillin-resistant *Staphylococcus aureus* (MRSA)

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Introduction: While *Staphylococcus aureus* specifically methicillin-resistant *S. aureus (MRSA* remains an important public health problem and a most common cause of clinical/nosocomial infections. Due to the emergence of MRSA, the multiple numbers of effective antibiotics with higher dosages is required and prescribed to treat the invasive MRSA infections which further deteriorate the current situation. Alternative approaches for prognosis and effective treatment against infection are required more than ever.

Purpose: The purpose of this study was to evaluate the effect of bioactive extracts from blueberry and blackberry pomaces and its major components against MRSA and rescue the inactive antibiotics supplemented with trace amount of bioactive berry pomaces extracts.

Methods: Two MRSA isolates, including one (C98) recovered from processed chicken meat and other one (COL, Sabath et al Ann N Y Acad. Sci. 1974, 236:435–43) as a reference strain, were used in this study. Antibiotic resistance of the MRSA was determined with agar dilution method as well as evaluated the presence or absence of specific antibiotic resistance genes by PCR. Synergistic or rescuing effect of bioactive extracts of berry pomace and antibiotics were checked in broth dilution methods.

Results: Bioactive pomace extracts of Blackberry (512 GAE µg/ml) and blueberry (256 GAE µg/ml) inhibited the growth of C98 and COL completely. Both MRSAs, C98 and COL, were resistant to a single or multiple antibiotics tested regardless the presence and absence of antibiotic resistance gene. The synergistic effect of bioactive berry pomace extracts and its major components with methicillin and erythromycin inhibited the growth of MRSA isolates and could reduce the MICs and make sensitive.

Significance: Bioactive extracts from berry pomace are potential alternative antimicrobials to treat MRSA infections and act synergistically with inactive antibiotics to reduce MIC significantly and make them further effective.

T7-03 Pathogenicity and Physicochemical Properties of *Campylobacter jejuni* Treated with Natural Phenolics from Industry Byproducts

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❖ ***Developing Scientist Competitor***

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

Purpose: The purpose of this study was to investigate the phenotypic and genotypic alterations of *C. jejuni* exposed to both lethal and sub-lethal concentrations of berry phenolic extracts.

Methods: Bacterial growth pattern, cell surface hydrophobicity, auto-aggregation capability were determined. Adhesion and invasiveness assay was carried out in cell-culture model. Expression of virulence genes was determined with qRT-PCR. *In vivo* experiment was carried out in a-day-old chick model.

Results: Minimum bactericidal concentration of blackberry and blueberry pomace extracts (BPE) were 0.8 and 0.6 mg GAE/ml on *C. jejuni*. Treatment with sub-lethal concentrations of BPE altered the cell surface hydrophobicity, auto-aggregation capacity, and cellular motility, significantly. Interaction of *C. jejuni* with cultured host cells were altered significantly; BPE reduced bacterial invasion into human intestinal epithelial cells (INT407) and in chicken fibroblast cells (DF1) by > 0.5 logs, compared to the control. Differential expression of *perR, flvA, sodB, fdxA, cmeB, cmeC,* and *clpA* genes in *C. jejuni* were observed, due to treatment with BPE. In chicken model, 1.0 mg GAE/ml BPE as water supplement reduced the natural colonization of *Campylobacter* in chicken cecum by > 5 logs compared to the control group.

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

T7-04 Antimicrobial Activity of N-Halamine Coated Materials in Broiler Chicken Houses

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Introduction: Broiler chicken houses are usually at a high risk of microbial cross-contamination. Newly released Food Safety Modernization Act (FSMA) regulation emphasizes the role of production procedure: the initial load of foodborne pathogens may significantly affect the safety of final products.

Purpose: To maintain an optimal hygiene condition, N-halamines are considered as ideal antimicrobial materials due to their superior antimicrobial efficiency against a broad spectrum of bacteria, low toxicity, high stability, rechargeability of antimicrobial activity, and low cost. Our purpose in this study was to investigate the antimicrobial activity of 1-chloro-2,2,5,5-tetramethyl-4-imidazoidinone (MC), a non-bleaching N-halamine compound, and its coated materials in broiler chicken houses.

Methods: The storage stability and the antimicrobial longevity of MC coated materials such as galvanized metal, aluminum, woods, etc. were studied. We used iodometric/thiosulfate titration to assess the chlorine content, and the "sandwich test" method to test the antimicrobial activity over a four-week period.

Results: Results showed that 0.04% MC in aqueous solution were able to kill *Salmonella* Typhimurium and *Campylobacter jejuni* completely at 10^6 CFU/ml within 30 min. Moreover, 1% MC treated samples possessed high antimicrobial activity and were able to kill *Salmonella* Typhimurium and *C. jejuni* completely in 2 h. The antimicrobial activity of MC treated materials remained up to 4 week, and the active chlorine atoms in the treated materials decreased from the initial 10^{16} atoms/cm^2 to 10^{15} atoms/cm^2.

Significance: Our results suggest that MC has a great potential application as a novel antimicrobial agent in broiler chicken houses during production.

T7-05 Commercially Available Citrus-based and Quillaja Extracts against Tulane Virus

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Introduction: Human noroviruses cause a great number of gastrointestinal illnesses and deaths annually. In the absence of human norovirus vaccines, alternate preventive and control strategies are being studied. Tulane virus (TV) has been recognized as a novel cultivable surrogate to determine inactivation efficacy against human noroviruses. Natural plant components that contain bioflavonoids and organic acids (e.g., citric acid) and quillaja bark extracts (QE) that contain quillaic acid glycosides and saponins are known to have antimicrobial effects. QE has previously shown antiviral effects against Aichi virus. The application of these extracts against TV needs to be investigated.

Purpose: The objective of this study was to determine the effect of a commercially available citrus-based formula (CX) and quillaja bark extracts (QE) on the infectivity of TV at room temperature (RT).

Methods: TV (200µl) at 7 log PFU/ml was mixed with equal volumes of undiluted citrus-based antimicrobial (CX), quillaja extract (QE), or water (control) for 30, 60, 120 and 180 min at room temperature. After each time point, treatments were stopped in cell-culture media supplemented with 10% fetal bovine serum and serially diluted in cell-culture media supplemented with 2% fetal bovine serum. Infectivity was measured using standard plaque assays on confluent LLC-MK2 cells in 6-well plates in comparison to controls. Each treatment was replicated thrice and assayed in duplicate and data were statistically analyzed.

Results: Treatment with CX at RT resulted in TV reductions of 0.75±0.07, 1.04±0.24, 2.40±0.1, and 2.97±0.15 log PFU/ml after 30, 60, 120 and 180 min, respectively, while QE (50% diluted in water) showed only 0.98±0.10 log PFU/ml reduction after 180 min.

Significance: The results indicate that the commercial citrus-based formulation is a promising alternate control strategy against TV. Further studies with CE using carrier tests and organic load against TV are needed for application as routine sanitizing washes.

T7-06 Antiviral Effect of Neutral Electrolyzed Water against Human Norovirus

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Introduction: Human norovirus can persist in the environment for weeks, and contaminated surfaces often serve as reservoirs for its transmission. Many conventional sanitizers and disinfectants have shown poor efficacy against this virus, and there is a need for validation of novel products with higher efficacy.

Purpose: To evaluate the anti-noroviral activity of neutral electrolyzed water (NEW) in both suspension and on surfaces.

Methods: NEW was generated using a Mini-UL-75a device (*Clarentis Technologies*, Palm Beach Gardens, FL). Human norovirus GII.4 Sydney 20% fecal suspensions (with and without an additional 5% organic load) were used as inoculum. Suspension assays (ASTM method E1052-11) were performed using NEW at concentrations of 50, 150, and 250 ppm free available chlorine (FAC, pH 7) with a 1-min contact time. Carrier tests (ASTM method E1053-11) were done using inoculated stainless steel coupons exposed to NEW (250 ppm FAC, pH 7) for 10, 15, and 30 min. Log reduction of norovirus genomic copy number as a function of NEW exposure was determined using RT-qPCR with and without prior RNase treatment.

Results: In suspension assay, NEW (250 ppm FAC, pH 7) produced a 4.8 ± 0.6 log GII.4 genome copy number reduction. An additional 5% organic load significantly reduced NEW efficacy, as evidenced by log reduction of 1.9 ± 0.2 (P<0.05). For surface tests, NEW (250 ppm FAC, pH 7) produced a 1.6 ± 0.7, 2.4 ± 0.5, and 5.0 ± 0.5 log reduction after 10, 15, and 30 min, respectively (P<0.05). Anti-noroviral efficacy decreased significantly to <0.2 log genomic copies in the presence of 5% higher organic load regardless of the contact time (P<0.05).

Significance: Under the parameters of this study, NEW shows promise as an alternative surface disinfectant when used at 250 ppm on clean surfaces for a contact period of 15-30 min.

T7-07 Control of Bacterial Foodborne Pathogens on Fresh Produce: A Trojan Horse Tale

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Introduction: Fresh produce is increasingly implicated in foodborne outbreaks. Most fresh produce are consumed raw emphasizing the need to develop non-thermal methods to control foodborne pathogens. Temperate bacteriophages are found within genomes of various bacterial foodborne pathogens. These prophages can be induced, resulting in lysis of their host.

Purpose: The goal of this study was to investigate induction of prophages as a novel strategy to control bacterial pathogens on fresh produce.

Methods: Growth of various lysogenized foodborne pathogens exposed to a phage inducer (0 or 2 µg/ml mitomycin C) was monitored by OD_{600}. Release of induced prophages from their host was confirmed by PCR to detect phage-specific integrases. The ability of mitomycin C to induce prophages in *Escherichia coli* O157:H7 and *Salmonella Duesseldorf* on fresh produce was evaluated by inoculating the stem scar of red greenhouse tomatoes or spinach leaves with 5×10^7 and 5×10^8 cells, respectively. After drying, mitomycin C (6µ/ml) was sprayed onto each sample, while control samples were sprayed with water. Following overnight incubation, the bacterial cells were recovered and plate counts were performed.

Results: Beginning at 3 hours after addition of mitomycin C, growth of *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* strains resulted in a marked decrease in OD_{600}. PCR confirmed release of prophages in these lysates. Growth of certain strains, including *Salmonella Tyresoe* shown to contain no phages, resulted in only a minor decrease in OD_{600} after addition of mitomycin C, suggesting lack of induction. A three-log reduction in *Salmonella Duesseldorf* and *E. coli* O157:H7 was observed on tomatoes sprayed with mitomycin C compared to those sprayed with water, while a one-log reduction in *E. coli* O157:H7 was obtained on spinach.

Significance: These findings serve as a proof of concept demonstrating that prophage induction can efficiently control bacterial foodborne pathogens on fresh produce.

Technical

T7-08 From the Microtiter Plate to the Industry: Application of the Bioprotective Concept in the Fresh and Minimally Processed Vegetables Industry

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Introduction: *Listeria monocytogenes* is ubiquitous bacteria that can be isolated from fresh and minimally processed vegetables. Thus the fresh cut vegetable industry is searching for natural treatment that ensures the safety of the product and fulfill consumer demands for foods with no preservatives.

Purpose: The purpose was to select culture(s) with anti-*listeria* activity, to validate their efficiency and neutral taste when sprayed on vegetables and to apply them in an industrial environment.

Methods: Around 1,500 lactic acid bacteria were tested in laboratory conditions for their anti-*Listeria* effect. Further tests (bacteriocin and acid production, taxonomic identification via DNA sequencing, safety verification, etc.) were performed on the most promising candidates. Then the selected bacteria were sprayed onto green salad and challenge tests against cocktails of *Listeria monocytogenes* were performed. Metagenomics study was also performed.

Results: The *Lactobacillus curvatus* allowed inhibiting the growth of *L. monocytogenes* during the shelf life (13 days) of the green salad stored at cooling temperature. In the control batch, the pathogenic bacteria growth potential was 1.4 log unit. During this test, no taste deviation was reported. Metagenomics results have shown a good survival and a strong dominance of the protective strain. In addition of the strong ability to compete for space ("jameson effect") this unique *Lactobacillus curvatus* is able to produce bacteriocin. For industrial application, a device was designed to spray a solution of bacteria, using water as carrier. The goal was to guarantee an optimal distribution of bacteria on the leaves while adding a low quantity of water (around 0.8%) not having any effect on the humidity or water activity of the product.

Significance: The study has brought a laboratory concept to an industrial application, already used in Europe by several manufacturers to prevent the growth of *L. monocytogenes* in their products.

T7-09 Molecular and Physio-Morphological Characterization of Novel Bacteriophages Targeting Diverse Strains of Biofilm-forming Shiga Toxigenic *Escherichia coli*

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) has emerged as a major group of foodborne pathogens with ruminants as host-reservoirs. Implicated in approximately 73,000 illnesses each year in the United States, human infections by STEC have been associated with a variety of foods. It is therefore crucial to devise effective control strategies. Use of target-specific and ubiquitous bacteriophages can be an attractive alternative to control STECs in the food industry.

Purpose: Molecular and physio-morphological **c**haracterization of STEC-infecting bacteriophages isolated from beef cattle operations in Oklahoma.

Methods: Bacteriophages specifically targeting biofilm-forming STEC (O157:H7, O121, O111, O103, O26, O145, O45) were isolated from environmental samples. Morphological features of isolated phages were examined under transmission electron microscope (TEM). In vitro inhibition assays were performed to measure the degree of STEC-lysis caused by phages. Viability of selected bacteriophages was tested at various pH and temperature ranges. One-step growth-kinetics assays were performed to measure the eclipse-period, latent-period, and burst-size of each phage. Phage adsorption-rate and optimal lysis time was also determined. Bacteriophage DNA was extracted using phenol-chloroform method and digested using restriction enzyme. The digested fragment was cloned and transformed into *E. coli* XO-1-Blue and sequenced subsequently.

Results: Several phages (*n*=52) showing inhibition towards the STEC serotypes were isolated. Phages were classified into *Myoviridae, Siphoviridae,* or *Tectiviridae* family, using TEM. All phages were resistant to temperature of up to 60°C and survived acidic (2, 5) and alkaline (7, 9, 11) pH ranges. Phages had a short latent-period (~20 mins), with large burst-size (41-67 virons/infecting cell), indicating high lytic activity. Genome sequencing revealed that the tail proteins of isolated phages were closely related to enteric phages specific to *Salmonella*, *Vibrio*, *Shigella*, and *Yersinia.*

Significance: Characterization of selected bacteriophages, targeting diverse strains of biofilm-forming STECs, would help with the development of effective bio-control strategies with potential uses in the food industry.

T7-10 Characterization of Antimicrobial Properties of *Salmonella* Phage Felix O1 Embedded in Low-density Continuous Xanthan Coatings on Poly(lactic acid) Films

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Introduction: Salmonellosis is one of the top three most common and serious forms of foodborne illness globally. While protocols are in place to sanitize meat products, accidental incidental contamination at points throughout the production process still remains a problem. Food packaging is an important component of preventing food borne illness by protecting products from external contamination and inhibiting growth of pathogenic and spoilage bacteria already present. However, conventional packaging is susceptible to protective failure enabling the development of spoilage and serious foodborne illness.

Purpose: The purpose of the study was to develop and evaluate the efficacy of a *Salmonella* Felix O1 bacteriophage coated PLA packaging film to control the growth of *Salmonella* spp. on surface of meat.

Methods: Concentrated *Salmonella* phage Felix O1 was incorporated into a thin layer of xanthan polymer adhered to PLA film. *Salmonella* growth on both agar and beef surfaces in contact with this phage coated film was compared with control films over time.

Results: *Salmonella* growth was significantly inhibited in the presence of the bacteriophage coated packaging film both on plates ($P<0.05$) and in semi-solid culture growth curve assays ($P<0.05$). No inhibition was evident from the control groups. Recovery of phage released from coating showed less than one-log reduction in titer from input bacteriophage concentrations. Preliminary analyses show more than a one-log reduction in *Salmonella* persistence in surface contaminated meat at 10°C over three days following contamination when wrapped aerobically with the phage coating, relative to phage free control xanthan coatings, for an overall 10^3 fold reduction.

Significance: Similar approaches could be used to develop packaging films inhibitory to diverse foodborne pathogens and spoilage bacteria, thereby significantly reducing the incidence and severity of these pathogens in addition to extending product shelf life.

T7-11 Evaluating Antibiotic Resistance Genes in Soils with Applied Manures

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Introduction: Antibiotics are commonly used in livestock production to promote growth and combat disease. Recent studies have shown the potential for spread of antibiotic resistance genes (ARG) to the environment following application of livestock manures.

Purpose: In this study, concentrations of bacteria with ARG in soils with applied poultry litter (PL; 2 years) or from a beef cattle backgrounding operation (BB; 3 year) were determined.

Methods: Samples were taken (1) following PL application to soils under conventional or no till management and (2) from soils taken from the BB while livestock were on-site and following their removal. Microbial populations with genes conferring resistance to tetracycline (*tetQ* and *tetW*), erythromycin (*ermB* and *ermF*) or sulfonamides (*sulI*), were quantified using quantitative, real-time (qPCR) analysis.

Results: In soils with applied PL, concentrations of ARG for sulfonamide and tetracycline resistance increased up to 3.0 orders of magnitude (OM; mean concentrations 2.6 to 6.9 x 10^8 copies g^{-1}) following PL application but were near background by the end of the season. Concentrations of bacteria with AR genes were highly variable across the BB landscape, but in general initial concentrations averaged between 2.5 and 3.3 OM higher in the dirt congregation areas than in grassy areas. The highest concentration of ARG remaining in those soils (2.1±3.2 x 10^9 copies g^{-1}) and background in grass (4.8±3.5 x 10^6 copies g^{-1}) were for sulfonamide resistance.

Significance: These results suggest that the concentration of bacteria with ARG significantly increase in soils where manures are deposited but levels are mitigated by time and landscape management. Future research should determine which AR populations remain in soils and the potential for transmission among microbial populations in the soil.

T7-12 Antimicrobial Resistance in *Salmonella* Isolated from Food Animals at Slaughter by the Food Safety Inspection Service, USDA

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Introduction: In an effort to better understand antimicrobial resistance (AMR) in food-producing animals, in CY 2013, the Food Safety and Inspection Service (FSIS) partnered with the Food and Drug Administration (FDA) under the National Antimicrobial Resistance Monitoring System (NARMS) to undertake a new cecal sampling program. This serves as a complement to FSIS's existing Pathogen Reduction/Hazard Analysis and Critical Control Point (PR/HACCP) system sampling program.

Purpose: Cecal sampling provides insight into AMR in food animals without the influence of slaughter and processing interventions. Comparison with PR/HACCP provides valuable understanding of AMR persistence from pre-harvest through slaughter and processing.

Methods: Samples were collected from establishments nationwide with frequency determined by slaughter volume and analyzed for microbial targets including *Salmonella*. Antimicrobial susceptibility testing was performed by broth microdilution and minimum inhibitory concentrations were interpreted using Clinical and Laboratory Standards Institute (CLSI) criteria when available. Described here are results from resistant *Salmonella* isolated from cecal (CY2014 *n*=362, CY2015 *n*=330) compared to those from PR/HACCP (CY2014 *n*=266, CY2015 *n*=636) for CY 2014 and 2015. Due to sampling design differences, a test of statistical significance was not applied.

Results: Among resistant isolates from both cecal (C) and PR/HACCP (H), few were resistant to azithromycin and ciprofloxacin (<2% [C] and <1% [H]). Ceftriaxone resistance was lower in cecal than in PR/HACCP in both 2014 (11.9% [C] and 18.8% [H]) and 2015 (11.8% [C] and 14.3% [H]). Streptomycin resistance, mostly attributed to chicken, was also found less frequently among resistant cecal isolates (< 54%) than in PR/HACCP (> 75%). Distribution differences by region and establishment size are further described.

Significance: AMR differences were observed not only between cecal and PR/HACCP but also among commodities sampled. AMR in isolates from PR/HACCP may be influenced by in-plant processing factors, while resistance among cecal isolates may reflect on-farm antimicrobial usage.

T8-01 Evaluation of the Implementation of a Food Safety Intervention for Food Pantries

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Introduction: Food insecurity affects approximately 14.5% of the U.S. population. In North Carolina an estimated 2,500 emergency food providers provide a regular, local source of sustenance some partnered with nationally-sponsored food banks or as independent, community-driven entities. Food pantries in North Carolina exist outside of inspection requirements.

Purpose: A set of evidence-based guidelines was created and implemented in food pantries using outreach and extension principles and this research evaluated the guidelines for effectiveness.

Methods: Using the Theory of Planned Behavior as a framework, a pre- and post-intervention evaluation was executed. On-site managerial interviews and observations based on a modified standardized inspection were carried out in 60 urban, rural, and suburban food pantries in 12 North Carolina counties that met the study criteria. A randomized sample of 30 food pantry managers was provided an online intervention designed to provide reasoning and strategies for reducing foodborne illness risk. A difference-in-difference model was used to examine if the provision of online information improved food safety behaviors.

Results: Two hand-hygiene related actions (no bare hand contact with ready-to-eat foods and handwashing sink availability) revealed statistically significant differences for pantries that are food bank partners ($P<0.10$) compared to independently-operated sites. Food bank partners were also likelier to have and use thermometers in the refrigerators and freezers ($P<0.01$). For those who definitively viewed the online guidelines, the managers were more likely to have requirements for their food suppliers and regularly provide recall information.

Significance: Though they worked in the past, online food safety guidelines do not work for all populations. The results reveal that there remains room for improvement in the provision of food safety information, as the pantry managers had little incentive to view the guidelines online. Without operational change, the food pantries will continue to indulge in risky behavior that effects a vulnerable population.

T8-02 School Responses to Norovirus Outbreaks: Policies, Procedures and Potential for Improvement

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Introduction: Norovirus is estimated to be the leading cause of viral gastroenteritis in school settings and the implementation of effective outbreak control and management measures are needed to limit virus transmission.

Purpose: The objective of this study was to investigate and examine policies and practices of schools during norovirus outbreaks, including outbreak control and communication methods, through school decision makers and sanitation staff.

Methods: Norovirus procedures were collected via a convenience sample of 92 North Carolina school principals. Participants were asked a combination of open-ended, Likert scale, and importance-ranking tasks regarding school policies during a norovirus outbreak, including cleaning measures, exclusion policies and communication with parents and students. Additionally, twenty school janitors were interviewed about their knowledge of norovirus control measures and specific school practices for dealing with norovirus.

Results: Only 13% of principals knew detailed school norovirus control policies and 42% indicated that janitorial staff would be a better source of that information (n = 60). Additionally, 30% of principals stated their school had no written policy on staff exclusion during an outbreak and 18% had no student policy (n = 72). Sixty-six percent of principals said written policies for janitorial staff would be helpful, and 60% indicated a need for better resources on GI illnesses (n = 70). Interviews with janitors revealed that the effectiveness of quaternary ammonium compounds on norovirus is not well understood.

Significance: This is one of the first studies to analyze school practices during a norovirus outbreak and results suggest that there are important gaps that need to be addressed in school procedures. There is a need for written exclusion and control guidelines within schools and norovirus specific cleaning protocols that identify appropriate sanitizers, concentration and contact time.

T8-03 Evaluation of a Reusable Learning Object for Educating Undergraduate Students about Good Manufacturing Practices

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Introduction: Undergraduates are the future workforce leaders, so it is critical to equip them with food safety competencies. Experts throughout industry believe the skill undergraduates lack the most is applying theoretical knowledge to real world problems. There is a projected 10% increase in food safety employment between the years 2010-2020. The development of asynchronous virtual reusable learning objects (RLOs) has potential to increase the efficiency of and/or complement existing methods for teaching undergraduates food safety concepts.

Purpose: This project evaluated the "The Plant Tour GMP Game," a reusable learning object designed to teach students Good Manufacturing Practices in the context of a virtual food processing facility, and its effect on participant's knowledge of GMPs, attitudes toward food safety, and normative, behavioral, and control beliefs before and after playing the game.

Methods: Students from 42 food science clubs around the United States were contacted to participate in this study. The participants (n=44) were asked to take a pre-survey, play the game, and then take a post-survey. All questions asked in the pre-survey were also asked in the post-survey, and additional questions about the game design were added to the post-survey.

Results: Overall, "The Plant Tour GMP Game" is an effective teaching tool for undergraduate students. There were significant knowledge gains (P<0.05) for seven out of the ten knowledge questions. The number of participants who agreed or strongly agreed that they could identify GMPs in processing plants significantly increased (P<0.05) from 39% to 84%. The component of the game participants liked most was the videos (n=11).

Significance: Participants knew more about GMPs and felt more comfortable identifying GMPs after playing "The Plant Tour GMP Game." This game could be a useful teaching tool to implement into undergraduate food safety courses by multiple instructors at different institutions.

T8-04 Food Safety in Ontario High School Students: Knowledge, Attitudes, and Practices

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Introduction: High school youth represent a unique audience for interventions aimed at improving safe food handling and preparation. They are the age cohort immediately prior to the 'second weaning' phenomenon, a hypothesized increase in foodborne illness that occurs when those in their early 20s are cooking for themselves for the first time. They are also at an age when food preparation practices may not be fully established, such that appropriate teaching of safe food handling at this age may help instill lifetime safe food handling habits.

Purpose: The objective of this research was to determine the food safety knowledge, attitudes and practices in high school students in Ontario, Canada.

Methods: We administered a school-wide paper survey to the student body (n=2,860) of four Ontario (Canada) high schools, and conducted an in-depth survey and observed a recipe preparation in Grade 10 and 12 food and nutrition classes in these schools (n=8 classes; n=119 students), to assess knowledge, attitudes, and self-reported and actual practices. This study was approved by a University of Waterloo Research Ethics Committee.

Results: Although food safety knowledge was generally low, most students felt confident they could prepare safe, healthy meals. Observed practices were not ideal. For example, although 77% of all students reported 'always washing hands with soap/running water after handling raw meat,' only 26% were observed doing so. The most common method of checking chicken doneness was by time (47%).

Significance: Food safety knowledge and behaviors are poor, yet confidence in preparing safe, healthy meals is high, among high school students. Because work and volunteer opportunities put students in contact with both the public and food, this group is important to target for increased safe food handling education.

T8-05 Assessing the Usage of Food Thermometers at College Football Tailgates

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Introduction: Temporary and informal food production settings such as festivals, community gatherings and tailgates have little infrastructure for safe food handling practices. Many commercial and volunteer-driven outdoor temporary events have been linked to foodborne illness outbreaks, but little is known about safe food handling practices of small groups and individuals in tailgate settings.

Purpose: The goal of this project was to evaluate current food thermometer usage at college football tailgates, using a mixed-methods approach of observation and interviews. Additional aims were to engage with tailgaters around safe food handling, distribute food safety materials to individuals who may not have them, and evaluate usage.

Methods: Trained data collectors from five U.S colleges engaged with tailgaters to collect data on thermometer usage. Participants were asked about their thermometer usage, and from those who use a food thermometer, more specific information was collected on their personal use (n=173). Tailgaters who responded that they did not use a thermometer were given a meat thermometer, apron, and food safety information (n=350).

Results: A total of 33.1% of tailgaters responded that they already use a food thermometer, and 66.9% responded that they do not (N=523). When told they could select all that apply, respondents who use thermometers reported using them on beef (72.6%), pork (69.7%), chicken (64.6%), fish (16%), other (10.9%), and eggs (5.1%) (n=173). When asked to choose one option that showed how often they use their thermometer, participants responded with all the time (34.3%), usually (31.4%), occasionally (24.6%), and rarely (9.7%) (n=173).

Significance: The results provide insight on the need for food safety and training and specific education for tailgaters. Targeting education efforts to this group can aid in reducing the risk of foodborne illness at temporary food settings such as tailgates.

T8-06 Use of Focus Groups to Assess Consumer Knowledge and Behaviors Related to Safe Handling of Mechanically Tenderized and Enhanced Beef Products

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Introduction: Shiga toxin-producing *Escherichia coli* [STEC] outbreaks associated with beef have been attributed to industry and consumer mishandling. While cooking and handling instructions are required on labels, the frequency of use and effectiveness of these messages are unknown. Mandatory labeling of mechanically-tenderized [MB] beef products begins in 2016. Consumer knowledge of what MT beef products are and how to safely handle them is unknown.

Purpose: The objective of this study was to assess consumer knowledge about what MT beef products are and how they are/should be handled.

Methods: Sixteen focus groups were conducted in Virginia and North Carolina. Participants (n = 62) were asked about their beef purchasing, handling, and preparation choices and behaviors. In addition, their understanding of, attitude towards, and handling of MT beef was assessed. Finally, participants brainstormed ideas for how safety information could be effectively delivered to consumers.

Results: Despite the safe handling instructions included on packaging, participants were unable to properly identify correct cooking instructions. All participants were aware they should use a thermometer, but only three actually did. Cooking experience and personal preferences were cited as reasons for not using thermometers. Color was the main indicator of doneness, followed by touch, and time. Only two participants had knowledge of MTB; but, when prompted with descriptors, participants generally recognized previously purchasing MTB products. The primary theme from focus groups was that participants wanted more labeling and information. Upon learning more about MTB, participants were divided upon whether the knowledge would affect purchasing decision; but, noted its importance. Participants stressed the importance of word choices, honesty, and saliency in message delivery.

Significance: These findings can be translated to develop more specifically targeted methods of safe handling communications to the public through understanding of their preferences. In a future study, intervention methods will be developed and evaluated.

T8-07 Evaluation of How Different Signs Affect Poultry Processing Employees Hand Washing Practices

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Introduction: Signs can provide repetitive training on specific food safety practices for food processing multicultural employees. From observation, posted signs for workers in many food processing facilities tend to be text-heavy and focus specifically on occupational hazard safety.

Purpose: The purpose of this study was to evaluate the effectiveness of newly-developed hand washing pictograms on employees' hand washing behavior using video observation.

Methods: Five employee hand washing behaviors (soap use, wash completeness, wash time, complete rinsing, and towel use) were evaluated with (a) no intervention, company signs posted and considered the baseline; and compared to (b) hand washing behavior after experimental hand washing signs were displayed (short term and long term) at a raw poultry slaughter facility (Facility A) and a poultry processing facility (Facility B).

Results: A total of 894 hand washing observations were counted from a total of 53.5 hours of video recorded. Both facilities showed a significant increase (P<0.05) in soap use after the new sign was introduced at both short and long term time periods. There was a significant increase (P<0.05) in washing, time, and rinsing observed by Facility B employees, when baseline data was compared to the short term. This indicates that a new sign could increase hand washing compliance. Sign color also had a significant effect (P<0.05) on employee behavior for washing and time of washing, while behavior for four of the five variables was significantly different on days of observation.

Significance: While signs can be a useful tool to offer as recurring food safety training for food processing employees, employees tend to revert back to old habits after a short period of time.

T8-08 Good Research is Not Sufficient for Food Safety Innovation – The Role of Networks, Innovation System Conditions and Intermediaries

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Introduction: Scientists frequently assume that research is required for food safety innovation, to the exclusion of the other conditions required for innovation. Innovation systems theories attempt to define the components and conditions required for innovation. Actors are a key system component.

Purpose: To explore the effects and roles of various actors in a food safety innovation system.

Methods: The Australian red meat food safety innovation system was studied by conducting an on-line survey of participants in 41 past projects conducted by Meat & Livestock Australia, measuring innovation, actor involvement and effectiveness and conditions defined by two theories arising from sectoral and technological innovation systems literature. The study was guided by case study methodology, supplemented with fuzzy-set Qualitative Comparative Analysis and standard statistical techniques.

Results: Two hundred seventy survey responses were received. In this innovation system, projects with high membership in the innovation outcome set were likely to involve a larger number of actors than projects with low set membership. Researcher involvement and effectiveness was scored highly across all projects, so were not a significant factor in determining the innovation outcome. The involvement and effectiveness of actors such as industry firms, industry associations, government, the intermediary organization orchestrating the project was significantly greater ($P<0.05$) in projects with high innovation set membership than those with low membership. The development of a network of actors does not, by itself, lead to favorable innovation system conditions. There is no single defined configuration of actors that leads to innovation system conditions being met or to particular types of innovation outcome.

Significance: Innovation system theory provides policy-makers with guidance to create an environment that allows food safety innovation to occur. Management of projects is needed to ensure that innovation system conditions are met, and ensure that research meets stakeholder needs and is put into practice.

T8-09 Recipe Modification Improves Food Safety Practices during Cooking of Poultry

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Introduction: Many consumers do not practice proper food safety behaviors when preparing food in the home, resulting in the possible spread of foodborne pathogens. Several approaches have been taken to improve food handling among consumers, but a deficit still exists in actual practice of appropriate behaviors.

Purpose: This study assessed whether the placement of food safety instructions within recipes for chicken breasts and ground turkey patties would improve consumers food safety behaviors.

Methods: A total of 186 consumers were observed while preparing a baked parmesan sesame chicken breast and a mushroom turkey burger following recipes that either did or did not contain specific food safety instructions. Additionally they were interviewed about the perceived burden of the experience.

Results: Participants who followed recipes with food safety instructions demonstrated significantly improved food safety behaviors as they prepared their items. Only 22 to 59% of those who did not have the safety instructions washed their hands at appropriate times when preparing the breasts, as compared to 63 to 90% for those who were told to do so in the recipe. With the exception of washing hands after placing patties in the pan (79% for both groups), handwashing percentages were also lower (20 to 39%) for the group without safety instructions (20 to 39% vs. 53 to 63%) than for the group with the instructions. Food thermometers were used by 85% of those who were told to do so, in comparison to only 20 to 30% of those who followed recipes without the safety information. The majority of consumers thought the recipes with the embedded instructions were easy to use, and that they would be likely to use similar recipes at home if available.

Significance: This study shows that recipes could be a good source of food safety information for consumers, resulting in the potential to improve food preparation behaviors and reduce foodborne illness.

T8-10 Knowledge and Risk Communication for Undercooked Oyster Preparation in Restaurants

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Introduction: Restaurants serving oysters often communicate risks associated with consuming raw shellfish, although there may be a lack of communication about the risks of consuming steamed oysters. In 2009, over 400 patrons of a North Carolina restaurant acquired norovirus from lightly steamed oysters. Oysters must be cooked to an internal temperature of 145°F for 15 seconds to ensure the kill of *Vibrio spp*. However, oysters must be cooked to an internal temperature of 194°F for 1.5 minutes to inactivate norovirus.

Purpose: It is necessary to determine the level of food safety knowledge and communication within restaurants to ensure that customers are provided enough information to make educated decisions about consuming undercooked oysters.

Methods: Twenty-six restaurants serving oysters in an urban center were interviewed in person or by phone call. The interviewers visited or called each restaurant location unannounced with the hope of receiving unbiased survey answers. Individuals that were interviewed at the restaurant locations included chefs, managers, owners, greeters, and wait staff.

Results: Of the 26 restaurants serving oysters, 27% served only raw oysters, 4% served only steamed oysters, 69% served both raw and steamed oysters. Restaurant personnel at 47% (n=19) said that if a customer asked whether steamed oysters are fully cooked, (defined as free of all foodborne pathogens) they would respond, "yes," while 52% said "no" or that it depended on certain parameters. Of the 26 restaurants that served raw and/or steamed oysters, only three restaurant personnel identified a foodborne pathogen associated with oysters.

Significance: This study demonstrates that there is a disconnect between restaurant employee food safety knowledge specific to the safety of steam oysters. This information provides the foundation for an educational intervention for managers, servers, and patrons as it relates to risk management practices.

T8-11 You Say Tomato, I Say Raw Agricultural Commodity: Effectively Communicating Regulatory Requirements to Produce Farmers

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Introduction: There are approximately 121,000 fruit and vegetable farmers in the United States. Considering exclusions and exemptions in the Food Safety Modernization Act Produce Safety Rule, about 35,000 of these farmers may require produce safety training and the rest may need training to meet market demand for food safety practices. The Produce Safety Alliance standardized training includes both best practices and regulatory requirements to reduce produce safety risks, however, the balance between regulatory language and understandable terms was difficult to strike.

Purpose: This narrative case study provides insights and mechanisms used to balance the need to retain the value of the curriculum to enhance produce safety training while meeting regulatory requirements.

Methods: Seven training modules were developed over five years, with input from hundreds of subject matter experts including farmer focus groups. Upon incorporation of the final Produce Safety Rule language, the modules were submitted to FDA for review. Comments from the first round of the final FDA review were summarized according to type.

Results: One challenging training module, agricultural water, received 380 comments during the review. In all, 50% (209 of 421) of the statements in the training module received comments. Approximately 8% questioned the accuracy of the regulatory information and 9% represented requests to convert grower-friendly language to regulatory language. The majority of comments (74%) addressed training material that was not intended to represent the regulatory language but may have been interpreted as regulatory content.

Significance: The educational value of the module was retained by arranging the slides into two types: those dealing with plain language Good Agricultural Practices, and the other dealing with regulatory requirements that included the addition of the provision symbol in the corner of the slide. This strategy resulted in a well-balanced module and is presented as a practical model for other food safety education professionals facing similar challenges.

T8-12 Identifying Unique Nutrition and Cooking Skills among Northern Maryland Residents

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Introduction: Safe food handling messages by the government, academia and non-profits are shared to benefit the health and wellbeing of all consumers. The health literacy of consumers affects their ability to understand and successfully use that information. Those possessing an inadequate level of health literacy may be more at risk of acquiring a foodborne illness than those with adequate health literacy.

Purpose: Focus groups were held with Maryland community members to identify what food safety and nutrition messages were misunderstood among high and low health literacy groups.

Methods: Participants were prescreened and sorted into high and low health literacy groups. High health literacy was defined as working in the health profession or self-identification as a caretaker of someone in a vulnerable population. A total of six focus groups were held; three were classified as high health literacy and three were classified as low health literacy. Focus group discussions were transcribed and analyzed for emergent themes between and within groups.

Results: Emergent food safety themes discussed by both literacy groups were: herbs and spices, chemicals/pesticides, trust of food handlers, Thanksgiving food preparation, washing raw poultry, and storage/leftovers. Unique topics among the high literacy groups were the lack of home economics classes and low knowledge regarding organic food safety. Unique topics among the low literacy groups were low cooking literacy and self-efficacy, as well as a desire to understand the science behind specific recommendations.

Significance: This study demonstrates similar and different gaps in food safety and nutritional knowledge among high and low health literate groups. Community cooking classes should be offered to provide hands-on n education that can help reinforce food safety and nutritional messages. Catering cooking classes to both high and low health literate groups can address knowledge and behavior barriers in a safe learning environment.

T9-01 Frequency of Resistance to Antimicrobial Agents among MRSA Strains Isolated from Broilers and 'Pluck Shop' Workers in Trinidad

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of zoonotic infections in many countries. People with occupational contact with food producing animals are at high risk of colonization. In Trinidad, MRSA strains have been reported in cattle, humans and pigs, however, it has not been documented in broilers.

Purpose: The aim of this study was to determine the prevalence of MRSA and the frequency of antimicrobial resistance in MRSA isolated from broilers and workers at the 'pluck shops' in Trinidad.

Methods: Choanal, cloacal and pharyngeal swabs taken from broilers and nasal swabs from humans were enriched in Mueller Hinton broth with 6.5% sodium chloride followed by secondary enrichment in phenol red mannitol broth with 75 mg/L aztreonam and 5 mg/L ceftizoxime. Enriched samples were plated on both CHROMagar MRSA and Brilliance MRSA. Suspect isolates were identified as *Staphylococcus aureus* (SA) using standard biochemical procedures, then confirmed as MRSA using the PBP2a test kit and PCR for the *mec*A gene. Susceptibility of MRSA isolates to 16 antimicrobial agents was detected by the disc diffusion method.

Results: Of the 287 broilers and 47 humans sampled, MRSA was isolated from 11 (3.8%) and 1 (2.1%), respectively. All isolates were resistant to one or more of the 16 antimicrobial agents and all were susceptible to vancomycin, rifampicin and chloramphenicol. The frequency of resistance ranged from 25.0% (streptomycin) to 100% (oxacillin, penicillin and ampicillin).

Significance: The study demonstrated that slaughtered broilers and workers at 'pluck shops' in Trinidad harbor multidrug resistance MRSA. This is of public health significance as occupational exposure of humans can lead to an increased risk of acquiring MRSA infections.

T9-02 Prevalence and Genetic Characteristics of *Escherichia coli* Isolates from Slaughterhouses and Farms in South Korea

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❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* is normal inhabitant in animal intestine. Certain serotypes of pathogenic *Escherichia coli* are recognized as an important cause for diarrhea, and verotoxin-producing *E. coli* is considered zoonotic foodborne pathogen.

Purpose: The objective of this study was to investigate the prevalence of *E. coli* in the slaughterhouses and farms in S. Korea, and the genetic properties for the isolates were determined.

Methods: Samples were collected from the carcass (n=28) and feces (n=29) of cecum in six slaughterhouses, and from soil (n=20) and feces (n=63) in 20 cattle and pig farms in S. Korea. The samples were plated on *E. coli*/Coliform count plate incubated at 35°C for 24 h. Isolated colonies on the plates were identified by 16s rRNA analysis. To detect virulence genes in all *E. coli* isolates, PCR were performed using the primers specific to pathogenic genes (*stx1*, *stx2*, *eaeA*, *inV*, *aggR*, *lt*, and *st*) and to determine O antigen serotype. Antimicrobial susceptibility for the isolates were also examined. In

addition, pulsed-field gel electrophoresis (PFGE) digested with *Xba*I restriction enzymes was conducted to compare the genetic correlations among the isolate.

Results: Of 140 *E. coli* isolates, 13 samples [9.3%; one feces and one carcass samples (3.5%) in slaughterhouse and 11 feces samples (13.3%) in farms] were verotoxin producing *E. coli* [*stx1* (1), *stx2* (9), and *stx1*+*stx2* (3)]. Genetic characteristics for other pathogenic genes (*eaeA*, *inV*, *aggR*, *lt*, and *st*) and antimicrobial susceptibility were varied among the isolates. In PFGE patterns of *E. coli*, geographical correlation was observed, but low genetic correlation was observed among serotypes.

Significance: These results indicate that there is high prevalence of verotoxin producing *E. coli* in cattle and pig farms in S. Korea, and they have geographical correlations.

T9-03 Prevalence of Extended Spectrum β-Lactamase-producing Bacteria and *Escherichia coli* O157:H7 on Commercial Beef Cattle Farms in North Florida

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Introduction: According to the Food and Agriculture and World Health Organizations, the primary route of transmission of antibiotic resistant organisms from the environment to humans is through food. It is generally accepted in the scientific community that the emergence of antibiotic resistant bacteria is due to the use of agricultural growth promotors in animal feed however; levels of antibiotic resistant bacteria continue to rise in food-producing animals not previously exposed to antibiotics.

Purpose: The purpose of this study was to determine the prevalence of extended spectrum β -lactamase-producing (ESBL) antibiotic resistant bacteria and *E. coli* O157:H7 in animal and environmental sources on commercial cow-calf operations in North Florida.

Methods: Over 1,000 animal and environmental samples were collected from 17 commercial beef farms across North Florida. ESBL-producing bacteria were enumerated from samples by plating onto MacConkey Agar supplemented with 4 µg/ml of cefotaxime. *E. coli* O157:H7 was enumerated from samples by plating onto sorbitol MacConkey agar supplemented with cefixime and tellurite.

Results: The average prevalence of ESBL-producing bacteria and *E. coli* O157:H7 on farms was 4.3 and 4.0 log CFU/g, respectively. Levels of ESBL producing bacteria were significantly higher in soil samples (P=0.002) and forage samples (P=0.03). Prevalence of *E. coli* O157:H7 was highest in forage samples with a concentration of 5.6 CFU/g (P=0.01). For both ESBL-producing bacteria and *E. coli* O157:H7, prevalence was lowest in water samples with a concentration of 1.2 CFU/ml and 0.5 CFU/ml, respectively.

Significance: These results suggest that transmission of antibiotic resistant organisms to food-producing animals may occur through environmental sources, specifically soil and forage. In addition, emergence of antibiotic resistant bacteria may occur naturally in the environment.

T9-04 Microbiological Quality Assessment and Validation of Peroxyacetic Acid, Lactic Acid, Lactic and Citric Acid Blend, and Sodium Hypochlorite against *Salmonella* on Broiler Carcasses and Wings Processed at a Small USDA-Inspected Slaughter Facility in West Virginia

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❖ ***Developing Scientist Competitor***

Introduction: Raw poultry products produced by small processors and sold within state are of particular food safety concern due to the exemption of USDA-FSIS poultry products inspection act.

Purpose: This study aims to evaluate the microbiological quality and the efficacy of antimicrobials to inactivate *Salmonella* on broiler carcasses and wings processed at a small USDA-inspected facility in WV.

Methods: Study I, 25 broiler carcasses were rinsed, pre-enriched in buffered peptone water (BPW), enriched in Rappaport-Vassiliadis medium and streak-plated onto XLT-4 and HardyCHROM™-agar. Presumptive colonies of *Salmonella* were confirmed by API-20E kits and qPCR (InvA gene). Aerobic plate counts (APCs), *E. coli*/Coliforms, and Yeast/Molds were analyzed on petrifilms. Study II, broiler carcasses and wings were spot inoculated with a two-strain mixture of *Salmonella* Typhimurium and Tennessee (5.7 log CFU/ml of sample rinsate), and then undipped, or dipped into peroxyacetic acid (PAA; 1,000-ppm), lactic acid (LA; 5%), lactic and citric acid blend (LCA; 2.5%), and sodium hypochlorite (SH; 70 ppm) for 30 s. Surviving bacteria were recovered in BPW (60-s shake) and spread-plated onto tryptic soy agar, XLT-4 and HardyCHROM™-agar. Data (2 replicates/3-4 samples/replicate) were analyzed using the ANOVA of SAS.

Results: APCs, coliforms, and Yeast/Molds were 2.62, 1.08, and 2.37 log CFU/ml on broiler carcasses, respectively. Thirty % (8/25) and 40% (10/25) of carcasses were positive for *Salmonella enterica* spp. and generic *E. coli* (0.48-1.70 log CFU/ml), respectively. Fewer (P<0.05) *Salmonella* were on broiler carcasses and wings treated with all antimicrobials with the fewest pathogen recovered on PAA treated carcasses (3.79 log CFU/ml) and wings (4.67 log CFU/ml). Reduction of *Salmonella* increased in the order SH (0.87 log CFU/ml) ≤ LCA (0.97 log CFU/ml) < LA (1.23 log CFU/ml) < PAA (1.52 log CFU/ml).

Significance: Results suggested the high percentage of *Salmonella* on locally produced broiler carcasses, and indicated that applying post-chilling antimicrobial dipping treatments could be a potential intervention approach to control *Salmonella* on locally raised/processed broilers.

T9-05 Impact of Dry Chilling on the Genetic Diversity and Survival of Naturally Occurring *Escherichia coli* on Beef Carcasses

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Introduction: Generally *Escherichia coli* and related mesophilic pathogens like *E. coli* O157:H7 do not grow at temperatures < 7°C. Thus *E. coli* is frequently used as an indicator organism to test effectiveness of dry chilling at small beef packing plants.

Purpose: To examine the effect of dry chilling on the genetic diversity of naturally occurring *E. coli* population on beef carcasses and on the survival of selected *E. coli* genotypes.

Methods: Carcasses selected at random were sampled for isolation of *E. coli* at various intervals for up to 67 h of a commercial chilling process. *E. coli* isolates were genotyped using multiple-locus variable-number tandem-repeat analysis (MLVA) and genetic diversity was estimated by isolate/genotype ratio. Survival of ten *E. coli* genotypes found only at 0 h (G1) and appeared at <8 h plus 8 or 24 h (G2) were examined after exposure to 75 and 100% relative humidity (RH) at 0 or 35°C for 67 h.

Results: A total of 254, 49, 49, 51, 23, 20 and 4 *E. coli* isolates were obtained at 0, 1, 2, 4, 6, 8 and 24 h, respectively. No *E. coli* was recovered at 67 h. Genetic diversity of isolates obtained between 1 and 24 h ranged from 1.33 to 1.88. All selected genotypes from G1 and G2 were completely inactivated at 75% RH and 35°C. Inactivation of *E. coli* genotypes from G1 and G2 were not significantly (P>0.05) different by exposure to 75% RH at 0°C and to 100% RH at 0 or 35°C.

Significance: The findings indicate that dry chilling may not impose selective pressure on the survival of *E. coli* on beef carcasses. Dry chilling could be used as a chemical free option to improve the microbial quality of meat at smaller packing plants.

T9-06 The Use of Novel Prevalence Calculation Methods To Estimate Pathogen Prevalence in Raw Ground Beef and Beef Manufacturing Trimmings Regulated by the Food Safety and Inspection Service

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Introduction: FSIS developed a novel method that incorporates its verification testing results and improved volume data to estimate the prevalence of *Escherichia coli* O157:H7, non-O157 Shiga toxin-producing *E. coli* (STEC) and *Salmonella* sp. in raw beef products. FSIS has defined prevalence as the proportion of 325-gram beef sample units that would test positive for a bacterial pathogen if the entire United States production were sampled and analyzed during a specified time period.

Purpose: To estimate pathogen prevalence in raw ground beef and beef manufacturing trimmings (MT43 and MT60 sampling projects, respectively) from raw beef producing establishments using fiscal year 2015 sampling data.

Methods: FSIS collected and analyzed ground beef (MT43) and beef trim (MT60) samples for each pathogen. To estimate pathogen prevalence, the annual production data (average daily volume x production days per month x 12) generated for each establishment by FSIS inspection personnel were used. This avoided stratifying samples into volume groups. Calculations took collected sample weights into account. Prevalence was then calculated as (sum(contaminated volume))/(sum(total volume)).

Results: FSIS analyzed 11,682 MT43 samples collected from 1,180 establishments and 3,277 MT60 samples collected from 426 establishments. Prevalence of *E. coli* O157:H7 and *Salmonella* sp. in MT43 samples was 0.05% (95% confidence intervals [CI], 0.01-0.20%) and 2.85% (95% CI, 1.79-4.52%), respectively. Prevalence of *E. coli* O157:H7, non-O157 STEC and *Salmonella* sp. in MT60 samples was 0.13% (95% CI, 0.02-0.81%), 0.47% (95% CI, 0.21-1.04%) and 1.66% (95% CI, 0.95-2.90%), respectively.

Significance: FSIS successfully developed and used novel prevalence calculation methods to estimate pathogen prevalence in regulated products. These estimates inform policies relevant to the prevention of pathogen contamination of raw beef products in FSIS-regulated facilities, and to monitor changes over time. This approach has been adapted for use in estimating pathogen prevalence and volume-weighted percent positive results in other regulated commodities (e.g., poultry products).

T9-07 Effect of Product Caliber Size and Fat Level on the Inactivation of *Escherichia coli* O157:H7 during the Manufacture of Dry Fermented Sausages

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❖ ***Developing Scientist Competitor***

Introduction: Dry fermented sausage (DFS) manufacturing processes that do not utilize heat have four other options, one being the demonstration of a 5-log reduction, which have been the subject of numerous validation studies. However, an area that can be studied in more depth is the effect of product caliber size and fat level on the inactivation of *E. coli*O157:H7 in DFS.

Purpose: Examine the effect of product caliber size, and fat level on the inactivation of *E. coli*O157:H7 during the fermentation and drying of DFS.

Methods: Three separate batches of sausage consisting of two fat levels (low, 17.5%; high, 25% w/w) and three caliber size casings (32, 55, 80 mm) containing a five-strain cocktail of *E. coli* O157:H7 at 10^7 CFU/g were manufactured. Sausages were subjected to fermentation and drying and were monitored for changes in *E. coli*O157:H7 numbers, pH, aw and M:P ratio over an 8-week period and the results were subjected to ANOVA for significance.

Results: A significant reduction in the inactivation time of *E. coli* O157:H7 was observed with an increase in product caliber size. Increasing casing diameter form 32 to 55 and 80 mm increased time for 5-log reduction from 32 to 39 and 53 days, respectively. Similarly, an increase in fat level leads to a significant increase in time for *E. coli*O157:H7 inactivation. For example increasing the fat level form 17.5 to 25.0% increased the duration form 32 to 46 days respectively. Shelf-stable M:P ratio of 1.3 was achieved on days 18, 25 and 53 for 32, 55 and 80 mm caliber size, respectively.

Significance: DFS products with higher fat level and larger caliber size will require longer processing times to achieve the 5-log reduction of *E. coli* O157:H7.

T9-08 A Majority of *Salmonella* Heidelberg Outbreak-associated Food Isolates Have Enhanced Heat Resistance

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* Heidelberg is the ninth most likely to cause invasive illness among the top ten *Salmonella* serovars in the United States. *Salmonella* Heidelberg is responsible for four recent outbreaks, including the Foster Farms outbreak from 2013 to 2014, which sickened and hospitalized 634 and 200 people, respectively.

Purpose: Due to scope of the Foster Farms salmonellosis outbreak, we hypothesized that food isolates associated with the outbreak have enhanced resistance to processing stresses such as heat and antimicrobials. The purpose of this study was to compare the heat resistance of nine isolates associated with the Foster Farms outbreak with other common serovars.

Methods: *Salmonella* Typhimurium 14028, *Salmonella* Heidelberg SL476 and nine food isolates representing six strains associated with the outbreak were heat shocked at 56°C to mimic hard scald during poultry processing. Aliquots were serially diluted and pour plated or spread plated at 0, 2.5, 5, and 7.5 min (log phase) and 0, 15, 30, 45, and 60 min (stationary phase); time-to-equilibrium was approximately 2.2 min. Statistically significant

differences between isolates and reference strains were determined by repeated measures analysis of variance (ANOVA) and Dunnett's test, with significance defined at $P<0.05$.

Results: There were no significant heat resistance differences among isolates in log phase cells; all but one isolate reached the detection limit by 7.5 min. During stationary phase, 6 of 9 and 2 of 9 outbreak-associated food isolates showed significantly increased heat resistance compared to *Salmonella* Typhimurium 14028 and *Salmonella* Heidelberg SL476, respectively ($P<0.05$). Final average survival ranged 0 to 3.3 log CFU/ml.

Significance: Increased heat resistance of many of the outbreak-associated food isolates may contribute to survival through processing (e.g., scald) or final cook steps. Further research is crucial for understanding the variation in heat resistance and other stress among and within common *S. enterica* serovars in order to improve food safety practices and regulations.

T9-09 Through-Chain Antibiotic Sensitivities of *E. coli* and *Salmonella* from an Australian Vertically Integrated Poultry Operation

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Introduction: Recently, there has been increased attention to the antibiotic resistance of foodborne pathogens, especially from poultry. Therefore, a leading Australian poultry producer initiated a survey to review its antibiotic usage and the level of resistance found in poultry-associated *E .coli* and *Salmonella*.

Purpose: The purpose of this study was to investigate the antibiotic resistance patterns of *E. coli* and *Salmonella* isolates from a vertically integrated poultry producing region in Australia.

Methods: *E. coli* and *Salmonella* were isolated from breeder and broiler farms (drag swabs, $n = 41$) and from corresponding dressed carcasses (whole carcass rinses, $n = 30$) at point of pack, using standard cultural techniques. The antimicrobial sensitivities of the isolates were determined using the Gram-negative veterinary and human antibiotic panels for the Vitek 2 system. The data was analyzed and compared to international studies to identify any significant antibiotic resistance patterns seen in this production area.

Results: Seventy-five percent of *E. coli* isolates ($n = 8$) were sensitive to all antibiotics tested, with one isolate resistant to both ampicillin and piperacillin, and one isolate tetracycline-resistant. Forty percent of *Salmonella* isolates from breeder ($n = 5$) and broiler ($n = 10$) flocks were sensitive to all antibiotics. However, 53% were resistant to piperacillin, 47% ampicillin-resistant, 40% nitrofurantoin-resistant, and 13% tetracycline-resistant. The majority of *Salmonella* isolates from carcass rinsates were primarily *Salmonella* subsp. II ser 1,4,12,27:b:[e,n,x], with 83% resistant to ampicillin, piperacillin and nitrofurantoin.

Significance: The low levels of resistance indicate that careful antibiotic use in the Australian poultry industry is having little impact upon circulation of antimicrobial resistance. Additionally, the pilot study data will facilitate a nationwide survey to assess the current prescribing practices and compare antimicrobial resistance to global data, thereby reducing the risk of foodborne antibiotic resistance spreading to the general public.

T9-10 Molecular Analysis of *Salmonella enterica* Strains Carried by Poultry Entering the Food Chain in Trinidad

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Introduction: Foodborne salmonellosis is a leading zoonosis impacting on public health in Trinidad and Tobago. *Salmonella* infections are usually associated with the consumption of contaminated food products and infections in humans generally lead to acute gastroenteritis that may be complicated by severe systemic sequelae depending on the strain, serotype and host-specific factors. Changes in agricultural practices and antimicrobial misuse in food producing animals may be accelerating factors for the evolution of more virulent and multidrug-resistant strains.

Purpose: The purpose of this study was to identify the virulence profile of *Salmonella* isolates from poultry sources and to investigate the occurrence of antimicrobial resistance and associated resistance genes.

Methods: A total of 1,000 cecal samples were collected from different farms and 'pluck shops' in Trinidad and screened for *Salmonella* spp. by isolation using standard techniques. All the isolates were confirmed to be *Salmonella* by biochemical tests and a genus-specific PCR. PCR-based assays were performed in 88 *Salmonella* isolates to detect 13 virulence-associated genes (*invA*, *pefA, pipA*, *sefC*, *spiC*, *fliC*, *sopE, mgtB, spi4D, pagN, viaB, shdA,* and *spvC*). The isolates were further assessed for their susceptibility to 12 antimicrobials using the disc diffusion method. Resistant isolates were subsequently examined for the presence of 11 resistance genes (*int1, qnrB, oqxA, oqxB, TEM, SHV, ampC, CTXM1, CTXM2, CMY2* and *Acc1*).

Results: Of the 13 virulence genes assayed, 11 (84.6%) were detected and their prevalence ranged from 1.3% (*sefC*) to 84.2% (*mgtB*). Only 4 (36.4%) of the 11 resistance genes tested for were detected and their prevalence ranged from 1.3% (Ampicillin) to 63.2% (Quinolones group). The frequency of multidrug resistance was 46.2 % as detected by the disc diffusion method.

Significance: The detection of several virulence in multidrug-resistant *Salmonella* isolated from poultry slaughtered at 'pluck shops' can be expected to pose potential health hazards to consumers.

T9-11 Rapid Systematic Review and Meta-analysis of Research on the Efficacy of Interventions to Control Nontyphoidal *Salmonella* spp. in Beef and Pork from Primary Production to Processing

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Introduction: Pork is one of the top sources of salmonellosis worldwide, while beef products have been implicated in numerous outbreaks. Interventions are needed during the production and processing of beef and pork to reduce *Salmonella* contamination and to protect consumers.

Purpose: The purpose of this study was to review the global body of evidence on interventions to control *Salmonella* in the beef and pork chains.

Methods: A rapid systematic review and meta-analysis of the global research literature was conducted to determine the efficacy of interventions to control *Salmonella* in beef and pork from primary production to processing. The review was conducted using the following steps: comprehensive search strategy, relevance screening of abstracts, relevance confirmation of articles, risk-of-bias assessment, data extraction, meta-analysis (where appropriate), and a weight-of-evidence assessment for estimates of intervention efficacy.

Results: A total of 520 relevant articles were identified (n=309 for pork; 216 for beef). On farm, several interventions strategies were effective to control *Salmonella* in pigs in various contexts, while only biosecurity measures were consistently effective in cattle. Limited evidence was found for transport and lairage interventions for both pork and beef. During processing, high confidence was found in the estimates of effect for pre-chill hot water/steam treatments and organic acid washes to reduce *Salmonella* prevalence on beef and pork carcasses, respectively. Other interventions consistently effective under commercial conditions included chemical hide washes of cattle, and scalding and singeing of pork carcasses. Overall, the

evidence supported enhanced reductions in *Salmonella* through a combination of interventions implemented concurrently or successively along the chain.

Significance: Various interventions can contribute to the control of *Salmonella* along the food chain continuum for beef and pork; the most appropriate combination of these should be guided by the local context and situation.

T9-12 Metagenomics of Spoiled Meat: Meet the Suspects

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Introduction: Meat and meat products are generally subject to fast spoilage. The huge variety in packaging and processing likely results in a variety in dominant spoilage flora as well. Metagenomics now enables analysis of the spoilage flora more cheaply and less labor intensive, inevitably leading to establishment of product segment associated microbiota.

Purpose: The purpose of this study is to analyze the dominant spoilage flora in several meat product segments.

Methods: We have applied metagenomics to several spoiled samples of vacuum packaged cooked meat, vacuum packaged fresh meat and vacuum and MAP packaged minced meat as well as roast beef. The V1-V4 region of the 16S rDNA gene was amplified, and high throughput sequencing was conducted with the Illumina Myseq. Typically 2000-3000 reads per sample were obtained.

Results: Vacuum packaged hot dogs (3 samples) contained a dominant flora of LAB like *L. sakei* and *Lc. mesenteroides* and was consistent with a previously established list of top 5 spoiling bacteria by culture dependent methods. Aerobically packaged hotdogs contained 70% of *Brochotrix*. Four vacuum packaged fresh beef pieces slaughtered in different countries and a piece of lamb meat, were invariably dominated by only *L. piscium* and *Lc. inhae*, not found in cooked meat. Both species contributed to 70% or more of the total flora. In the case of minced beef, the type of packaging seemed some but limited importance. This may be because the minced meat samples showed high bacterial counts from the start. Apart from four different species of LAB present above 10% in different samples, many samples contained at least 20% of *Photobacteria* and occasionally up to 70%, making it an important spoiler of minced beef.

Significance: A better understanding of the dominant spoilage flora will lead to better and specific interventions to elongate shelf life.

T10-01 Developing Methods to Identify Surrogates for *E. coli* O157:H7 in Validation of Fresh Produce Washing Processes

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Introduction: Cross-contamination during fresh produce washing is commonly prevented using chlorine inactivation treatment. Surrogate microorganisms can be used in validation of chlorine washing process. However, fresh produce washing incorporates physical, chemical, biological and kinetic factors which create an intricate process for which little is known regarding surrogate selection.

Purpose: The purpose of this study was to identify the important elements relevant to produce washing processes and identify methods that will be used in surrogate selection.

Methods: The behavior of three (3) non-pathogenic microorganisms (generic *E. coli* Nissle1917, *Pediococcus pentosaceus* and lettuce isolate 813-F1) were examined in comparison to *E. coli* O157:H7 based on phenotypic similarities. Chlorine inactivation kinetics of *E. coli* O157:H7 and the non-pathogenic strains were evaluated with varying pH levels (6.5 and 8.0) and exposure times (3-30 seconds). Detachment of leaf-bound *E. coli* O157:H7 and non-pathogenic strains at different inoculation levels (~2 log, 6 log) and drying conditions (aging time, temperature) in wash water was examined.

Results: Chlorine inactivation at pH 6.5 resulted in a range of viability corresponding to *E. coli* O157:H7 and the non-pathogenic strains; demonstrating a sharp inactivation curve for *E. coli* O157:H7, EcN and *P. pentosaceus*. Whereas, inactivation at pH 8.0 allowed more survival relating to exposure time for all microorganisms. Detachment from inoculated leaves at 2 log and 6 log inoculation showed steady survival levels in wash water at 0 ppm and significantly lower survival at 1 ppm for all strains excluding 813-F1; 813-F1 was consistently less chlorine-sensitive. Aging time was not seen to have remarkable effects on bacterial transfer during washing.

Significance: These results suggest assay methods of chlorine inactivation at pH 6.5 and detachment with 6 log initial inoculation may be useful in selecting appropriate surrogates for fresh produce washing.

T10-02 Elucidating Human Norovirus Attachment to the Surface of Strawberries

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Introduction: Fresh berries, including raspberries and strawberries, have been associated with human norovirus outbreaks. Although norovirus has been shown to bind to berries, the specific berry components that contribute to virus attachment are unknown. Candidate mechanisms for attachment include histo-blood group-like antigens (HBGAs) or other unknown ligands in berry tissue; native berry microflora; and/or electrostatic and hydrophobic interactions between fruit and virus.

Purpose: To identify strawberry characteristics and/or components which contribute to human norovirus attachment to berries.

Methods: Two cultivars of whole strawberries and berry pistils were collected. The binding affinity of human norovirus GI.6 and GII.4 (Sydney) to berry surface slices and pistils was determined by RT-qPCR-based suspension assays. Visual localization of GII.4 Virus-Like Particles (VLPs) on berry surfaces was performed by confocal microscopy, and VLP adherence to pistils was confirmed by transmission electron microscopy (TEM). To determine the specificity of norovirus interactions to pistils, zeta potential, carbohydrate composition, and carbohydrate linkage analyses were performed. Additionally, a lectin-binding competitive ELISA was used to determine if specific strawberry sugar moieties contributed to norovirus binding.

Results: Based on suspension assay results, >90% and >94% ($P< 0.05$) of input norovirus bound to strawberry surfaces and pistils, respectively. Fluorescently labeled VLPs on the strawberry surface were primarily located on broken pistils and achenes. TEM results showed VLP attachment on pistils, with aggregation occurring on disrupted pistil surfaces. Physiochemical analyses of pistils showed a highly electronegative zeta potential (pH range 3.5 to 9). Glucose, arabinose, and galactose were the primary terminal sugars on complex carbohydrates of strawberries. Exposing purified strawberry carbohydrates to lectins significantly reduced VLP binding affinity.

Significance: Human norovirus binds tenaciously to strawberry surfaces and pistils, particularly regions of injury. Results suggest that specific carbohydrates facilitate virus binding to strawberries and efforts to identify these ligands continue.

T10-03 Microbial Community Structure and Chemical Composition of Surface Waters: Implications for the Recreational Water Standards and Microbial Safety of Strawberries

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Introduction: The Produce Rule adopted current EPA recreational water standards where the geometric mean (GM) and statistical threshold value (STV) of samples are not to exceed 126 CFU/100 ml and 410 CFU/100 ml of generic *E. coli,* respectively. However, questions remain on the direct correlation of these parameters with the presence of fecal contamination in water and the potential pre-harvest cross-contamination of produce.

Purpose: To study the effects of sampling frequency, depth, and timing relative to disturbance events on the microbial structure and chemical composition of surface waters used for irrigation/frost protection of strawberries.

Methods: Six hundred 1G-water samples were collected during 1.5-years from a natural surface water reservoir used for irrigation/frost protection of strawberries. Thirty-six samples were collected every 15 days at 3, 6, 9 and 12 m from the inlet of the irrigation pump and at 3 depth ranges; 0.5-1, 1.75-2.25, and 3-3.5 m below the water surface. Samples were filtered (modified Moore swab) and the pellet was used to characterize the physico-chemical and microbiological composition/structure (generic *E. coli*, *Enterococci*, STEC, *Salmonella*, Bacterial-16S and Fungal-ITS regions) of water.

Results: Rain events significantly increased detection of *Salmonella*, STEC, generic *E. coli* and *Enterococci*. Sampling depth, 0.5 vs 2.5 m, had a significant effect on pH (6.95 vs 6.2), TOC (7.3 vs 5.1 mg/L-C), turbidity (10.4 vs 11.5 FTU), and on the microbiological diversity/structure, whereas distance from shore had no significant effects ($P<0.05$). The dominant microbial families between 0.5-2 m depths were *Pseudanabaenaceae, Nostocaceae* and *Synechococcaceae*, while between 2.5-3.5 m the dominant families were *Moraxellaceae, Aeromonadaceae, Methanosaetaceae* and *Pseudanabaenaceae*. All samples met the GM and STV set for generic *E. coli* by 2.5-fold, even in those where *Salmonella* and/or STEC were detected at multiple depths and during/after rain events.

Significance: Depth, sampling date, and rain events are the most important parameters when designing surface water monitoring plans in order to establish a baseline of microbial water quality.

T10-04 The Transfer of Generic *Escherichia coli* from Simulated Wildlife Feces to and Die-off on Field-grown Lettuce in New York State

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Introduction: Wildlife intrusion, untreated manure and contaminated irrigation water have been associated with pathogen contamination of produce. While past studies examined the prevalence of pathogens in wildlife, livestock and water, relatively few examined the mechanisms behind the transmission of pathogens to and survival on field-grown produce.

Purpose: This study was performed (i) to calculate the die-off rate for *E. coli* in feces and on produce under field conditions, and (ii) to calculate transfer coefficients for *E. coli* from feces to lettuce.

Methods: Two studies were conducted. In the first study, lettuce was inoculated with *E. coli*, and harvested 0-10 days following inoculation. *E. coli* concentration on the lettuce was determined and die-off rates were calculated. Correlation tests were used to examine the relationship between weather and die-off rate. In the second study, feces inoculated with *E. coli* were placed in a lettuce field 0-3 days before irrigation. After irrigation *E. coli* concentration on the lettuce and in the feces was determined, and transfer rates were calculated.

Results: In the first study the average daily die-off for *E. coli* on the lettuce over 10 days was 0.52 log MPN (Standard Deviation [SD] = 0.56). The only weather factor that was significantly associated with die-off rate was leaf wetness ($P < 0.002$). In the second study, 89% of the lettuce heads tested positive for *E. coli,* and, on average, 0.027% (SD= 0.17) of the *E. coli* present in the feces transferred to a given lettuce head.

Significance: These findings provide die-off and transfer rates for *E. coli* in feces and on lettuce, respectively, that can be used in quantitative risk assessments to help determine the level of risk of human illness from such events.

T10-05 Comparative Analysis of *Listeria monocytogenes* Strains from Outbreak Along with Those from Cantaloupe and Its Production Environment

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Introduction: *Listeria monocytogenes* is one of the most virulent foodborne pathogens, and the third leading cause of death among foodborne pathogens in the USA, with fatality rate up to 20%. Little is known about factors that might support the survival of *L. monocytogenes* in cantaloupe, which caused the worst listeriosis outbreak in the USA in 2011.

Purpose: This project was designed to identify the phenotypic and genotypic factors that support the survival and virulence of *L. monocytogenes*.

Methods: The genotypes and phenotypes of *L. monocytogenes* strains cultured from cantaloupe farm A, which were associated with the listeriosis outbreak and strains from farm B, which were not associated with any illnesses, were characterized using whole genomes sequencing and phenotypic microarray. Raw sequences were assembled using *de novo* approach and compared against one other. Phenotypic microarray results were analyzed using OmniLog software package with a cut-off value of ±3,000 OmniLog units.

Results: Genome sequencing analysis identified two unique regions (39 and 42 kbp) in the outbreak strain, in which over 60% of the genes were phage related. Function-based analysis revealed that the outbreak strain had 21 unique proteins, in particular 3 membrane transport proteins along with 2 invasion and intracellular resistance proteins. Phenotypic microarray analysis suggested that the outbreak strain had higher resistance to 3 osmolytes and 15 antimicrobial chemicals, whereas lower resistance to 11 pH conditions and 22 other antimicrobial chemicals along with lower growth in 11 carbon sources. The findings from phenotype comparisons appear to correlate well with genotype comparisons.

Significance: Comparative analysis of the genotypic and phenotypic characteristics will help elucidate mechanisms responsible for the survival and persistence of *L. monocytogenes* in food production environments, which may shed light on the development of effective preventive measures to control this pathogen in such environments.

T10-06 Evaluating Sanitation Treatments in Five New Jersey Tomato Packinghouses for Controlling Indicator Organisms

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Introduction: Information regarding the effectiveness of sanitizers in controlling pathogens and indicator organisms under real-world fresh produce packinghouse conditions is limited.

Purpose: The goal of this work was to quantify the effectiveness of sanitary treatments on indicator organisms, on tomatoes, in New Jersey packinghouses. Factors that influenced treatment efficacy were also examined.

Methods: Twenty samples of 5 tomatoes each were collected before and after sanitary treatment from 5 NJ packinghouses at 5 different times over a 3-year period (1,000 samples, 5,000 total tomatoes). Chlorine or peroxyacetic acid was applied to the tomatoes via a dump tank or spray bars. Treatment concentration varied between packinghouses and over the course of the study. Samples were plated on plate count agar and CHROMagar ECC to determine total plate, coliform, and presumptive *E. coli* counts. Colonies were enumerated and bacterial populations were expressed in log CFU/tomato.

Results: Packinghouse A had a higher percentage of *E. coli* positive samples (5-85%) after the sanitary treatment vs. before (0-20%). Packinghouse B exhibited the highest consistent mean reductions per trial for total plate count (0.35±1.00 to 1.67±0.91 log CFU/tomato) and coliform count (0.59±1.49 to 1.56±1.24 log CFU/tomato). Packinghouses C and E had consistently minimal total plate count reductions (0.45±0.73 to 0.74±0.89 log CFU/tomato and -0.1±0.50 to 0.75±0.64 log CFU/tomato, respectively). Packinghouse C had the least reductions in coliform count (-0.14±0.83 to 0.56±0.92 log CFU/tomato). Packinghouse D results were inconsistent with total plate count reductions ranging from 0.09±0.66 and 1.15±0.86 log CFU/tomato. Overall, statistically significant reductions occurred in only 2 or 3 out of 5 sampling times at each packinghouse.

Significance: Each of the 5 packinghouses used different sanitary procedures that resulted in a wide range of bacterial reductions. Our research suggests that sharing and standardization on a common set of best sanitary procedures may aid NJ packinghouses in achieving more consistent bacterial reductions.

T10-07 Synergistic Effect of Multiple Low-Dosage Chemical Sanitizers Used at Industrial Practical Treatment Times in Combination with Freezing against Foodborne Pathogens on Blueberries

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Introduction: Recent foodborne outbreaks in produce have obtained public concern and therefore it is essential to develop effective intervention methods to increase the food safety margin of produce.

Purpose: This study develops an efficient sanitization strategy by using multiple sanitizer "hurdles" [chlorine (Cl_2), aqueous chlorine dioxide (ClO_2), and lactic acid] at practical industrial treatment times in combination with freezing against foodborne pathogens on blueberries.

Methods: Wild blueberries were inoculated with bacterial cocktail (two strains for each pathogen – *Listeria monocytogenes* or *Salmonella* Typhimurium) by a dipping method. To investigate synergistic effect of chemical sanitizers, a conveyer belt equipped with double spraying system was developed to spray 150 ml of multiple chemical sanitizers [chlorine 100 ppm (Cl_2) + lactic acid (2%), aqueous chlorine dioxide 10 ppm (ClO_2) + lactic acid (2%), and chlorine 200 ppm (Cl_2) + aqueous chlorine dioxide 15 ppm (ClO_2)] on 25 g of blueberries and later berries were exposed 10 sec, 1 min, and 3 min industrial treatment times. Bacterial enumeration was conducted before and after freezing (at -17°C for 1 week).

Results: The efficacy of multiple sanitizer "hurdle" when combined with freezing increased significantly in inactivating foodborne pathogens. The double spraying treatment of chlorine and lactic acid in combination with freezing resulted in 6.8 log CFU/g (detection limit <1 log CFU/g) reduction of *L. monocytogenes* at 3 min contact time. Other double spraying combinations like chlorine dioxide + chlorine and chlorine dioxide + lactic acid with freezing resulted in > 6 log CFU/g reduction of *L. monocytogenes* at 3 min contact. *Salmonella* Typhimurium was reduced to 7.1 log CFU/g (detection limit < 1 log CFU/g) with double spraying chemical treatments such as chlorine + lactic acid and chlorine + chlorine dioxide, in combination with freezing.

Significance: This approach of multiple sanitizer "hurdles" together with quick freezing process is worth to be considered by frozen fruit industries.

T10-08 Minimal Thermal Treatments for Reducing Bacterial Population on Cantaloupe Rind Surfaces

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Introduction: Cantaloupe melon has been associated with outbreaks of foodborne illness due to consumption of contaminated fresh-cut pieces. Surface structure and biochemical characteristics of bacteria play a major role on how and where bacteria may attach and also complicates decontamination treatments and this has led to higher incidence of foodborne illness.

Purpose: In this study, we evaluated the effect of minimal thermal treatment using 3% hydrogen peroxide (H_2O_2) and water H_2O at 80°C to reduce microbial populations on cantaloupe rind surface and fresh-cut pieces.

Methods: Whole cantaloupes rind surfaces were inoculated with *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* at 4.5, 5.1 and 3.6 log CFU/cm^2, respectively and were stored at 5 and 22°C for 7 days before washing and minimal thermal treatment using 3% hydrogen peroxide (H_2O_2) and water H_2O. All treatments were for 300 s and the rind surfaces were examined with scanning electron microscopy and bacterial inactivation on a range of agar media.

Results: Efficacy of washing treatment in reducing attached bacteria and minimizing transfer to fresh-cut pieces were investigated at day 0, 3 and 7 of storage. Initial attachment was highest for *E. coli* O157:H7 and lowest for *L. monocytogenes,* but *Salmonella* exhibited the strongest attachment at all days tested. Washing with 3% H_2O_2 alone led to significant ($P<0.05$) reduction of bacteria and caused some changes in bacterial cell morphology. Bacterial inactivation on cantaloupe rind appeared to be dependent on duration of contact time. No bacterial pathogen was determined in fresh-cut pieces prepared from minimally heated 3% H_2O_2 and H_2O treatments including enriched fresh-cut samples. Microbial safety for all fresh-cut pieces from treated cantaloupes was established at day 6 of storage at 5°C and day 3 at 10°C.

Significance: Minimal thermal H_2O and 3% H_2O_2 treatment at 80°C of cantaloupes surfaces designated for fresh-cut preparation will enhance the microbial safety of fresh-cut pieces, and will drastically reduce the incidence of foodborne illness, and costly recalls of contaminated produce.

T10-09 Photodynamic Inactivation of *Salmonella* spp. on Fresh-cut Papayas and Their Physicochemical and Nutritional Quality Changes during 405 Nm Light Emitting Diode Illumination at Different Storage Temperatures

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Introduction: A 405 nm light emitting diode (LED) has recently paid the attention due to its antibacterial effect on foodborne pathogens in buffered solution or bacterial growth media. However, little research has been conducted on its effectiveness on foodborne pathogens and qualities on food matrix.

Purpose: The aim of this study was to determine the effect of 405 nm LED against *Salmonella* on fresh-cut papaya and to evaluate its impact on the physicochemical and nutritional qualities of illuminated papaya at different storage temperatures.

Methods: Each of *Salmonella* Agona, *Salmonella* Newport, *Salmonella* Saintpaul, and *Salmonella* Typhimurium was inoculated on the surface of fresh-cut papaya at the final concentration of 10^3 CFU/cm^2. The inoculated fruit was illuminated by LED for 24-48 h at 4, 10 or 20°C. The irradiance of LED was 18±2 mW/cm^2 and the distance between LED and the surface of papaya was 4.5 cm. Quality parameters of fresh-cut papaya were analyzed after LED illumination.

Results: Strong growth inhibition of *Salmonella* was observed by LED illumination at 4°C, resulting in continuous decrease to 1.6–1.9 log CFU/cm^2 for 48 h regardless of bacterial strains. Control populations increased to 3.8–4.6 log CFU/cm^2 at 10°C for 36 h, whereas LED-illuminated cells inactivated or inhibited to 1.5–3.0 log CFU/cm^2. *Salmonella* populations rapidly grew in controls to 7.5–8.4 log CFU/cm^2 at 20°C for 24 h, while LED-illuminated cells reached to 6.3–7.0 log CFU/cm^2, indicating the growth delay due to LED illumination. No significant difference in color, texture, antioxidant capacity, flavonoids, β-carotene, lycopene, and ascorbic acid was observed between LED-illuminated and non-illuminated fruits regardless of storage temperatures.

Significance: These results suggest that a chiller equipped with 405 nm LEDs could be used to preserve fresh-cut fruits at retail store, minimizing the risk of salmonellosis without deterioration.

T10-10 Factors Influencing the Formation of Conventional and Emerging Disinfection By-Products during Fresh-cut Produce Washing with Chlorine Sanitizer

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Introduction: Free chlorine is a widely used sanitizer in produce washing. A major drawback of chlorine is its potential to generate harmful disinfection by-products (DBPs). Recent research shows that chlorine may generate not only the conventional trihalomethane and haloacetic acid DBPs, but also emerging DBPs such as halonitriles, halonitromethanes, haloacetamides and nitrosamines, with the nitrogenous DBPs posing even higher toxicity potential.

Purpose: This study was to systematically evaluate how conventional and emerging DBPs may form under fresh-cut produce washing conditions and identify major factors that influence the DBP formation.

Methods: A variety of fresh-cut produce and organic matters extracted from produce were exposed to 0.5-200 ppm sodium hypochlorite (NaOCl) at different contact times (n > 50). The process water and produce were then analyzed for the occurrence of >40 different DBPs using three advanced analytical methods. The impacts of produce type, chlorine dosage, chlorine demand, contact time, reaction pH and temperature on DBP formation were systematically evaluated.

Results: The results thus far show that chlorine demand and DBP formation are highly dependent on the produce type and reaction conditions. Generally, higher total DBP concentration correlates with higher chlorine dose, longer washing time, lower pH and higher temperature, while the distribution among different DBP chemical groups may exhibit different trends. For example, strawberry exhibits greater overall DBP formation potential and faster reaction kinetics than lettuce, likely due to different organic precursor characteristics. Major DBPs include haloacetic acids, chloroform, chloroacetonitriles, chloroacetamides, chloral hydrate and chloropropanones.

Significance: Different organic matter from produce can react with chlorine to generate various DBPs. The yield and rate of DBP formation may vary considerably for different types of produce and washing conditions. A comprehensive understanding of these reactions will help development of safer chlorine sanitation strategies to minimize DBPs and associated risks.

T10-11 *Salmonella* Newport Interacts with Plant-derived Reactive Oxygen and Nitrogen Species on Tomato Fruit and Leaves

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Introduction: Plants recognize pathogens via pathogen-associated molecular patterns (PAMPs), mounting basal defense responses that are modulated by reactive nitrogen species (RNS) and reactive oxygen species (ROS). Plants are capable of recognizing enteric bacterial PAMPs, however the impact that the ensuing responses have on enteric bacterial colonization of plants is not known.

Purpose: The purpose of this project was to determine whether *Salmonella enterica* elicits generation of RNS and ROS in tomato seedlings and fruit, and in turn assess the effect of plant-derived nitric oxide (NO) and hydrogen peroxide (H_2O_2) on S. *enterica* populations.

Methods: Fruit of tomato cultivars (cv.) 'Nyagous', 'Money maker', and 'Heinz' and seedlings of 'Heinz' were treated with the NO scavenger 2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) or water (control), followed by *Salmonella* Newport or *Salmonella* Typhimurium inoculation, incubation, and retrieval for assessment of growth. To evaluate generation of ROS, 3 week old 'Heinz' leaves were syringe infiltrated with *Salmonella* Newport or water (control), and H_2O_2 production was measured through 3,3'-Diaminobenzidine (DAB) staining.

Results: Fruit of cv. 'Heinz' (P=0.034), 'Nyagous' (P=0.001) and 'Money Maker' (P=0.021) supported significantly higher *Salmonella* Newport populations on NO-scavenged tissue relative to control (n=5/treatment). 'Heinz' fruit showed suppression of growth earlier (24 hours post-inoculation (hpi)) compared to other cultivars (48 hpi). Significantly larger populations of *Salmonella* Newport (P=0.008), but not the *rpoS* partial mutant *Salmonella* Typhimurium LT2, were measured on NO-scavenged leaves of sterile 'Heinz' seedlings. ImageJ analysis of DAB staining revealed that *Salmonella* Newport infiltrated leaves produced significantly more H_2O_2 than the water control (P<0.001).

Significance: Cultivar-specific elicitation of NO and H_2O_2 in response to *S. enterica* was observed, with NO impacting *Salmonella* Newport populations on tomato fruit and leaves. This study suggests that *S. enterica* colonization triggers a basal immune response in tomato that restricts its growth in the tomato phyllosphere.

T10-12 Predicting Chlorine Demand of Fresh and Fresh-cut Produce during Washing

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Introduction: The presence of organic compounds can lead to a rapid depletion of free chlorine in produce wash water, which may compromise the microbicidal efficacy of chlorine-based sanitizers.

Purpose: This study was conducted to develop models capable of predicting chlorine demand for various fresh and fresh-cut produce wash water.

Methods: Ten fresh and fresh-cut fruit and vegetable simulated wash waters at different chemical oxygen demand (COD) were prepared. The chlorine demand and wash water quality parameters including pH, ORP, UV254, COD, turbidity, total protein content, total phenolic content and color difference between water and tested samples (ΔE) were measured. The correlations between variables were determined.

Results: The results shows that UV254 was most correlated with chlorine demand of various fresh produce (R=0.77) among all the tested parameters. Further analysis of chlorine demand and UV254 data shows two clusters exist: clusters for produce with high phenolic content and low phenolic content. The phenolic-to-protein/ΔE ratio (PPC) was created to identify which cluster each produce wash water belongs. Empirical models for predicting chlorine demand were developed as chlorine demand = 295.23 UV254 + 6.97, if PPC<0.6; or chlorine demand = 119.77 UV254 + 2.41, if PPC≥0.6. The validation results show that models can predict chlorine demand for the same produce tested at different COD as well as other produce that were not used for model development, with prediction error of 11.3 and 8.16 mg/L, respectively.

Significance: The results demonstrate that the predictive models developed using water quality parameters could be used to estimate the chlorine demand of different produce wash water.

T11-01 Global Food Attribution Estimates for 11 Major Pathogens for the Global Burden of Foodborne Disease Initiative

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Introduction: WHO has recently estimated that globally 600 million people contracted foodborne illnesses and 420,000 died in 2010. Food attribution estimates would help better target efforts to prevent these illnesses. Yet in many areas of the world information on the food sources of these foodborne illnesses is scant.

Purpose: We will report the results of an expert elicitation designed to provide globally consistent, and regionally specific food attribution estimates for 11 major pathogens. These attribution estimates were designed for use in the WHO work on the global burden of foodborne disease.

Methods: Expert elicitation is used because of a lack of consistent conventional data sources in many lower income regions. The study uses Cooke's Classical expert elicitation method because it provides a transparent, reproducible method of measuring widespread tendencies toward bias and toward over or understating objective uncertainty and uses this information in aggregating expert responses. Median food attribution estimates and 95% uncertainty bounds were developed for 154 pathogen/subregion pairs.

Results: Performance-weighted aggregate estimates provided more statistically accurate estimates than equal-weighted aggregates without great sacrifice of informativeness. Exposure to between 2 and 3 foods typically account for over 80 percent of total foodborne cases for specific pathogen/subregion pairs. The role of specific foods in causing foodborne illness due to specific pathogen varies across subregions for some, but not all pathogens. Uncertainty intervals are generally wide, as would be expected given scant data collection and research on food attribution in many regions of the world.

Significance: These estimates also provide a means of comparing the relative roles of different foods in causing major foodborne illnesses around the world. These results suggest that for many pathogens and subregions, it may be advisable to target prevention efforts on specific food exposure routes.

T11-02 Application of Bayesian Methods in Evaluating Trends in Foodborne Disease Outbreaks (1998-2014)

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Introduction: Understanding temporal trends in foodborne diseases is a major food safety and public health priority. Developing methods to analyze these trends is a challenge due to limitations such as sparse data and short periods for evaluation.

Purpose: We used novel Bayesian modeling techniques to produce more precise estimates of foodborne illness source attribution trends over time.

Methods: Two Bayesian statistical models were used to evaluate changes in the numbers of outbreaks associated with a set of food categories (e.g. meat, poultry, fruit, and vegetables) reported to the CDC Foodborne Disease Outbreak Surveillance System (FDOSS) for the years 1998-2014. The simple shrinkage trend model naively pools information from all food categories to estimate individual category intercepts and slopes; the latent cluster shrinkage trend model pools information from categories only to the degree that sets of categories appear to share a common change over time. These were compared with a fully stratified model, which effectively fits a separate intercept and slope model to outbreak counts for each food category.

Results: Both Bayesian methods produced more precise trend estimates than the fully stratified estimates; the latent cluster shrinkage model produced estimates with comparable shrinkage but greater precision than the simple shrinkage model. The median reduction in the width of the 95% credibility intervals (CI) of the slope estimates across the 14 food categories analyzed was 13% for simple shrinkage and 24% for latent cluster shrinkage. The mean reductions in widths were 22% and 29% respectively.

Significance: Both Bayesian models offered improved precision over a fully stratified model. Bayesian latent cluster shrinkage is a novel, more precise, and potentially less biased statistical method than full stratification or simple shrinkage for evaluating foodborne outbreak trends over short time periods with limited data.

T11-03 Foodbook: The Canadian Food, Water and Animal Exposure Study

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Introduction: One of the challenges facing Canadian enteric illness investigators in identifying the source of outbreaks has been the availability of exposure data for the Canadian population. Similarly, limited exposure data has impacted the ability to evaluate risks associated with enteric illness. Foodbook, a population-based telephone study that asked Canadians about their food, animal and water exposures was conducted in all provinces and territories to address this essential gap.

Purpose: The primary objective of Foodbook is to establish a database describing Canadians' exposure to foods and other exposures over a seven-day period that may serve as vehicles of enteric illness to inform timely and effective outbreak response. Foodbook will also support risk assessments to help target public health interventions to prevent illness, and inform food safety education initiatives and health promotion efforts linked to obesity and determinants of health.

Methods: A population-based telephone survey was administered using landline and cell phone sampling frames to randomly interview 10,942 Canadians across all provinces and territories over a one-year period. The sample was distributed evenly over time and four age groups (0-9, 10-19, 20-64, 65+). Foodbook collected data on exposures in the 7 days prior to interview (including food, animal contact, drinking and recreational water) and information on consumer food safety knowledge and practices, acute gastrointestinal illness, obesity indicators and demographic factors.

Results: Results will replace outdated and geographically disparate exposure information, and provide a more representative comparison group for Canadian outbreaks. Data on food, water and animal exposures were analyzed and compiled into the Foodbook Report, released in December 2015.

Significance: Foodbook will enhance the Canadian response to foodborne illness outbreaks, support risk assessments and public health interventions, and provide critical data to inform additional Canada-wide, cross-disciplinary efforts to prevent and control disease.

T11-04 Historical Indicators Associated with FSIS-Regulated Establishments Implicated in Outbreak Investigations, 2010-2015

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Introduction: In carrying out its regulatory mission, the United States Department of Agriculture Food Safety and Inspection Service (FSIS) performs numerous activities to verify food safety in federally-regulated meat and poultry establishments. These activities include plant inspections, food safety assessments, and in-plant pathogen testing. FSIS maintains records for these activities that can be used for fact-finding during outbreak investigations. In addition, product recalls and enforcement actions may result when an establishment has failed to comply with certain regulatory standards. To date, the extent to which an establishment's regulatory history is predictive of its role in a foodborne outbreak has not been extensively studied.

Purpose: To identify clues for use in the early detection of establishments responsible for outbreaks, we assessed the relationship between selected historical indicators and outbreak-involvement.

Methods: We reviewed FSIS investigation records for 2010–2015 and selected investigations associated with evidence sufficient to prompt a voluntary product recall. FSIS-regulated establishments implicated in these investigations ("cases") were then compared to similar ("control") establishments using a matched case-control design. Indicators assessed included history of inspection non-compliances, results of routine and non-routine food safety assessments, enforcement actions, product sampling positives, and product recalls.

Results: During 2010–2015, FSIS investigated 20 outbreaks conclusively linked to 22 FSIS-regulated establishments. Of these outbreaks, 12 (60%) involved *Salmonella* spp. and 8 (40%) involved Shiga toxin-producing *E.coli.* Implicated establishments will be described with respect to historical indicators, with comparisons made to control establishments.

Significance: Using historical data as evidence during outbreak investigations may improve the timeliness of control actions and by extension, reduce the number of illnesses associated with contaminated food. Such clues may also assist FSIS in identifying and directing regulatory resources to problem establishments in advance of an outbreak, resulting in illness prevention.

T11-05 Foodborne Outbreaks in Barbados (1998-2009): A Twelve-year Systematic Review of the Commonly Implicated Pathogens, Food Vehicles, Locations, Laboratory Detection and Quality of Epidemiological Investigations

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Introduction: Foodborne Disease Outbreaks (FBDOs) in the Caribbean are not just a public health issue, but they can have a major impact on the tourism industry. As the Barbadian economy derives a large part of its stability from tourism, characterization of FBDOs is important.

Purpose: The purpose of this study was to determine the aetiology, food vehicles, locations and peak seasons of foodborne outbreaks in Barbados, assess the quality of epidemiological investigations and report on laboratory proficiency.

Methods: We examined 24 foodborne outbreaks from a systematic review of published reports between 1998-2009 and public health laboratory data. Keyword searches included "foodborne," "outbreaks", "Barbados" and the inclusion criteria were: 1. Two or more cases of a similar foodborne disease occurring as a result of ingestion of a common food. 2. Confirmation by epidemiological and/or microbiological (laboratory) evidence. 3. Sufficient epidemiological evidence to support the occurrence of a FBDO in the absence of identification of a foodborne pathogen. 4. FBDO occurred between 1998-2009.

Results: There were 215 cases of illness and one hospitalization and 37.5% of outbreaks were associated with hotels/resorts; hotel-related outbreaks occurred mostly during June-September and non-hotel outbreaks, mostly in February. *Salmonella* Enteritidis phage type 8 (PT 8) was most commonly implicated, eggs and poultry were the primary vehicles. Laboratory surveillance revealed that there were 2331 cases of human gastroenteritis and *Salmonella* spp. (67.6%), *S. aureus* (14.9%) and *Campylobacter* spp. (11.8%) were most implicated. Three outbreak reports were assessed and received low scores when evaluated by five major criteria from the World Health Organization.

Significance: Reports indicated deficiencies in outbreak investigations and high levels of food contamination with indicator organisms suggesting that improvements in food hygiene and production practices were required.

T11-06 Differences in Foodborne Outbreak Risks by Preparation Setting, 1998–2012

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Introduction: National data on foodborne outbreaks can be analyzed to attribute foodborne illness risks to specific food sources. The characteristics of these outbreaks differ, however, by where the food was prepared, such as in a home, restaurant, or school. In particular, outbreak size and implicated foods can differ by preparation setting.

Purpose: In this Interagency Food Safety Analytics Collaboration study, we compared risk characteristics of outbreaks based on where the food was prepared (and often consumed).

Methods: Summary data on outbreaks caused by four key pathogens (*Salmonella*, *E. coli* O157:H7, *Campylobacter*, and*Listeria monocytogenes*) from 1998-2012 were collected from CDC's Foodborne Disease Outbreak Surveillance System (FDOSS). We characterized outbreaks by where food was prepared, such as homes, restaurants, churches, schools, nursing homes, and prisons. We examined differences in outbreak size, whether outbreaks occurred in a single state or multiple states implicated food sources, and other variables.

Results: Of 952 outbreaks included in our study, 27% implicated foods prepared in restaurants and 23% in private homes, with much less than 10% associated with fairs, picnics, churches, schools, nursing homes, or prisons. Over 8% had unknown preparation locations and 13% had multiple preparation locations. Food preparation location was a significant predictor of outbreak size ($p<.05$), and implicated food vehicles differed by preparation location. Furthermore, differences in preparation setting were observed for outbreaks occurring in a single state versus multiple states.

Significance: This exploratory analysis informed the development of a statistical model for outbreak-derived foodborne illness source attribution, in which food preparation location is one of four variables used to predict outbreak size. Understanding how risks differ by location of food preparation is important for interpreting attribution estimates and for targeting risk mitigation efforts to different audiences.

T11-07 *E. coli* and *Enterococcus* Contamination in Soil and Vegetables in Detroit Urban Gardens

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❖ ***Developing Scientist Competitor***

Introduction: Urban agriculture is gaining popularity worldwide. However, there are limited data on the microbial safety associated with urban agricultural production.

Purpose: To study the prevalence and antimicrobial resistance of *E. coli* and *Enterococcus* in soil and vegetables in three urban gardens around Metro Detroit area.

Methods: Soil and vegetable samples were collected from three urban gardens in Detroit from June to October 2015. A total of 15 soil samples and 48 vegetable samples (leafy greens and root vegetables) were collected. *E. coli* was isolated by enriching samples in Lauryl Tryptose Broth, followed by spreading on MacConkey Agar. *Enterococcus* isolation was carried out by a selective enrichment in enterococcosel broth followed by growing bacteria on enterococcosel agar. Bacteria were then identified by PCR and analyzed by disc diffusion for the antimicrobial susceptibility profile. All bacteria from unique soil and vegetable samples were examined for their susceptibility to ampicillin, chloramphenicol, ciprofloxacin, streptomycin, and tetracycline. In addition, ceftriaxone and trimethoprim/sulfamethoxazole were tested for *E. coli* and erythromycin and vancomycin were tested for *Enterococcus* as well.

Results: Out of 15 soil samples, 6 (40%) were positive for *E. coli* and 14 (93%) for *Enterococcus*. Twenty-three (48%) vegetable samples were contaminated by *E.coli* and 39 (81%) by *Enterococcus.* Garden variation was observed in the bacteria prevalence indicating possible impact of soil amendment on the microbial composition. Disc diffusion results on bacteria from unique samples revealed common streptomycin resistance (70%) in *Enterococcus,* intermediate resistance of *Enterococcus* to ciprofloxacin (70%) and erythromycin (65%), and intermediate resistance of *E. coli* to ampicillin (95%).

Significance: Microorganisms prevalent in urban agricultural production may serve as an important source of food contamination and antimicrobial resistance.

T11-08 Virulence Profiles and Conal Relationships of *E. coli* O26:H11 Isolates from Feedlot Cattle by Whole Genome Sequencing

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Introduction: STEC O26 is the second most important EHEC worldwide. O26 strains are characterized in two groups: EPEC O26 carrying LEE mostly causing milder disease and watery diarrhea, and STEC O26 that carry *stx* gene responsible for more severe outcomes. The *stx*-negative O26 can further be split in two groups. One O26 group differs significantly from O26 EHEC, whereby the other O26 EHEC-like shows all characteristics of EHEC O26, except production of STX.

Purpose: Although present worldwide, this serotype has been majorly described in Europe but little data about O26 molecular epidemiology is available for other continents thus it is unknown if these findings also apply for the United States. We wanted to determine the different populations of O26 *E. coli* present in US cattle.

Methods: We sequenced 42 O26:H11 strains from US healthy cattle (collected during 3 months in 2011) using MiSeq and compared them to O26:H11 genomes available at NCBI using a wgMLST analysis.

Results: We found as observed in Europe, O26:H11/H- in US cattle are highly diverse, with at least 5 different populations. Most strains were sequence type 29 (ST29). Two clear Lineages could be distinguished among ST29 strains isolated from cattle. Lineage 1 composed of O26:H11 EHEC-like (ST29) and O26:H11 EHEC (ST21), and Lineage 2 showing four diverse populations with different virulence content. Overall our analysis showed US healthy cattle carried pathogenic (ST21, *stx*1+, *ehx*A+, *tox*B+) and also potentially pathogenic (ST29, *ehx*A+, *tox*B+) O26:H11 *E. coli* strains. Furthermore showed that O26:H11 clinical strains from US belonged to a different clone (mostly ST21, *stx*1+, *ehx*A+, *tox*B+) than the newly emerged O26:H11/H- clone in Europe (ST29, *stx*1+ and/or *stx2*+, *ehx*A+, and *etpD*+).

Significance: Our results showed that whole genome sequence analysis is a robust and valid approach to identify, and genetically characterize *E. coli* O26:H11, which is of paramount importance for food supply safety and public health.

T11-09 A Large Scale Survey Describing the Relationship Between Different Animal Reservoirs and Human Campylobacteriosis

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Introduction: *Campylobacter jejuni* is a leading cause of bacterial foodborne gastroenteritis worldwide. *C. jejuni* contaminated poultry meat and meat products are considered the most important sources of disease in humans. Nevertheless, other animal reservoirs must be investigated to elucidate the part not attributed to poultry.

Purpose: The objective of this work is to describe using the comparative genomic fingerprinting (CGF) with 40-gene assay (CGF40) the *C. jejuni* population circulating in the poultry production chain in France and in different animal reservoirs to determine a link with human cases.

Methods: A total of 645 poultry isolates representative of the French poultry industry (farm, slaughterhouse and retail), 455 isolates from dogs and cats, 122 isolates collected from river water and shells and 143 strains from human campylobacteriosis were typed by CGF40 according to Taboada et al. (2012). Isolates were categorized into types based on more than 90% CGF40 fingerprint similarity (CGF-90%). The results were analyzed using Bionumerics software and the genetic diversity of the different strain populations was evaluated using the Simpson Index of Similarity (ID).

Results: A great diversity has been observed with 141 different types among the 1364 isolates typed (ID=0.958). Within the pet isolates (ID=0.895) and the clinical isolates (ID=0.910), the genetic diversity was significantly lower than among poultry isolates (ID=0.961) and water isolates (ID=0.961). The main part of human isolates (57%) were divided into 4 CGF-90% types all found in chicken and pet isolates. Moreover, few human isolates belong to CGF-90% types sharing by water isolates.

Significance: This is the first large scale survey in France involving representative number of isolates from poultry, pets, river water, shells and humans. The results confirm that poultry meat production remain a substantial source of human infections in France. But other animal reservoirs sources, especially pets, could be potential sources of campylobacteriosis.

T11-10 Temporal and Population Dynamics of *Salmonella enterica* ssp. *enterica* Serovar Agona Isolates from a Recurrent Multistate Outbreak

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Introduction: The largest outbreak of *Salmonella* Agona in the United States occurred in 1998. It affected more than 400 patients and was linked to toasted oats cereal. Ten years later, a similar outbreak occurred with the same outbreak strain linked to the same production facility.

Purpose: In this study, whole-genome sequence (WGS) data from a set of *Salmonella* Agona isolates were analyzed to provide insight into the evolutionary relationships among strains linked to two outbreaks of salmonellosis separated by ten years.

Methods: We analyzed WGS sequence data from 46 *Salmonella* Agona isolates. Five out of 46 isolates were associated with the 1998 outbreak and 25 isolates were associated with the 2008 outbreak while 16 isolates were unrelated to both events. We reconstructed a phylogenetic hypothesis of the samples using a reference-free k-mer based method for identifying variable sites. We then tested alternative hypothesis regarding differences in mutation rates and historical fluctuations in effective population size.

Results: Using SNP analyses, we were able to distinguish and separate *Salmonella* Agona isolates from both outbreaks with only a few SNP differences between them. The phylogeny illustrates that the 2008 outbreak involves direct descendants from the 1998 outbreak rather than a second independent contamination event. Estimates of historical fluctuations in population size for the whole dataset and one reduced to only containing the isolates associated with the outbreak showed that the latter had little to no change in effective population size. Further, there are fewer numbers of SNP differences within genes linked to cell mobility, intracellular transport, and transcription within the cereal clade.

Significance: Based on these results, there is evidence supporting the persistence of *Salmonella* over time with little genomic changes and that emerging lineages or clonal swarms may harbor a higher mutational load than observed in the larger population.

T11-11 Comparison of *Listeria monocytogenes* Invasion among the Serotypes Isolated from Foods and Human

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❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* is generally isolated from food, especially meat products, and 90% of the isolates are 1/2a, 1/2b and 4b. The pathogen causes foodborne disease by invasion intestinal tissue, and thus, they may have different invasion efficiencies among serotypes.

Purpose: The objective of this study was to evaluate the invasion efficiency of *L. monocytogenes* among serotypes, and between food isolates and human isolates.

Methods: To compare the invasion efficiency, *L. monocytogenes* serotypes (1/2a, 1/2b, 1/2c, 3b, 4b, 4d) isolated from foods (14 strains) and human (11 strains) were used. *L. monocytogenes* inocula were prepared in minimum essential medium (MEM) supplemented with 20% fetal bovine serum to obtain multiplicity of infection of 100. Monolayer (5×10^4 cells/ml) of Caco-2 cell grown in 24-well tissue culture plates was washed twice with phosphate buffered solution. One milliliter of the inocula were inoculated into the cell monolayer of Caco-2 cells, and they were incubated in 5% CO_2 at 37°C for 2 h. After treating Caco-2 cells with 50 µg/ml gentamicin and 0.5%Triton X-100, resulting suspensions were plated on tryptic soy agar with 0.6% yeast extract to enumerate infected *L. monocytogenes.*

Results: The invasion efficiencies of *L. monocytogenes* strains into Caco-2 cell were dependent on serotypes. However, the invasion efficiencies of *L. monocytogenes* food isolates were similar to those of *L. monocytogenes* human isolates.

Significance: This result indicates that different serotypes of *L. monocytogenes* may have different invasion efficiencies into Caco-2 cell.

T11-12 Study of the Potential Zoonotic Transmission of *Clostridium difficile* in Belgian Cattle Farms

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Introduction: Zoonoses are infectious that can be transmitted between animals and humans through direct contact, close proximity or the environment. Since domestic and food animals frequently test positive for the bacterium, it seems plausible that *C. difficile* could be zoonotic. A former study showed that the prevalence in veal calf aged less than 6 months was 22% while in adult cattle population, it was 6.9 %.

Purpose: This study aimed to determine the prevalence and the epidemiology of *C. difficile* in cattle farms and the possible spread of the bacterium among animals and farmers.

Methods: A total of 176 fecal samples of cattle were collected from 5 different Belgian farms (south East Belgium), from November 2015 to February 2016. A stool sample of each farmer was also requested. Detection of *C. difficile* was performed by classical culture on *C. difficile* selective medium (cycloserine cefoxitin fructose cholate). Isolates were characterized by PCR-ribotyping and Genotype Cdiff test (Hain Lifescience), which allows the detection of all toxin genes, mutations in *gyrA* gene and the deletion in the regulator gene *tcdC.* Toxic activity was confirmed by a cytotoxic assay on MRC-5 cells.

Results: *C. difficile* was detected in 14 of 178 (7.9%) animal samples. Isolates were grouped into five different types, including PCR-ribotype 015 (this ribotype is one the most encountered in hospitals in Belgium). The other types were UCL46A, UCL24*, UCL24, UCL33. All of them were identified as toxigenic by cytotoxicity assay and toxin genes profile. In contrast, none of the 5 farmers studied were positive for the bacterium.

Significance: Results obtained indicate that PCR-ribotypes commonly isolated from hospitalized patients are also present in cattle, indicating an animal reservoir. However, a zoonotic transmission could be not demonstrated in this preliminary study.

T12-01 Modeling the Inhibition of *Clostridium botulinum* in Reduced-sodium Pasteurized Process Cheese Products

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Introduction: The 1986 "FRI/Tanaka model" predicts safety of shelf-stable process cheese spread formulations using the parameters of moisture, pH, NaCl and disodium phosphate (DSP) to inhibit toxin production by *Clostridium botulinum*. Although this model is very reliable in predicting safety for standard-of-identity spreads, the effects of additional factors are not considered.

Purpose: To expand the *C. botulinum* food safety predictive model to consider the interactive effect of moisture, pH, potassium-based replacements for NaCl and DSP, fat, and sorbate.

Methods: Eighty formulations were identified using a central composite design targeting seven factors (50-60% moisture, pH 5.4-6.2, 0-0.2% sorbic acid, 10-30% fat, 1.7-2.4% NaCl, 0.8-1.6% DSP, and 0-50% potassium replacement for sodium salt). Treatments were inoculated with 3-log proteolytic *C. botulinum* spores (10-strain mixture) per gram, hot-filled into sterile vials, and stored anaerobically at 27°C. Samples (5/interval) were assayed at 0, 1, 2, 3, 4, 8.5, 17.5, 26, 40 and 56 weeks for presence of botulinum toxin using the mouse bioassay. A parametric survival model was fit to the censored time to toxin data. The model can predict both failure probability at specified times and time to toxicity with specified failure probability.

Results: All linear, quadratic and pairwise effects were considered for model fit. As hypothesized, the effects of pH, moisture, DSP, NaCl and sorbate were significant (P<0.001). Fat level and potassium-replacement were significant at P <0.017 and 0.053. The model is conservative, consistently predicting failure for toxic samples although it does predict failure for some samples that were not toxic. Comparison with previously collected challenge study data confirms that the model predictions are valid only for combinations within the ranges tested.

Significance: This research adds the factors of salt reduction, fat, and sorbate to the model predicting the botulinum safety of process cheese products. Additional study is required to expand the model to lower moisture and higher phosphate-emulsifier concentrations.

T12-02 Modeling Survival of *Salmonella* Enteritidis during Storage of Yoghurt at Different Temperatures

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❖ ***Developing Scientist Competitor***

Introduction: Yoghurt has an important role in the human diet due to its nutritional value and positive effect on health. This product has been recently included in a vulnerability assessments of food systems developed by Food and Drug Administration (FDA, 2012), suggesting that yoghurt could be a potential target for bioterrorist attack.

Purpose: To investigate the behavior of *Salmonella* Enteritidis in yoghurt at 4, 12, 20, and 25°C and develop predictive microbiology models for vulnerability assessment purposes.

Methods: Survival data were obtained at different temperatures by plate count method and used to fit survival models (Geeraerd model, Weibull model, the modified Weibull model, the trilinear model, the bilinear model) by using the package of nlsMicrobio in R software. To evaluate the effect of storage temperature on kinetic parameters such as inactivation rate (k_{max}) and shoulder (S_l), secondary models were developed by using two empirical models.

Results: According to the survival curves and smaller goodness of fit indices (RMSE, ACC_f), Geeraerd model with shoulder and tailing was selected as the most appropriate model to describe the survival of *Salmonella* in yoghurt during storage at different temperatures. At 4°C, *Salmonella* displayed the lowest inactivation rate (0.05 h^{-1}), whereas at 25°C, the maximum temperature assayed, it showed the highest inactivation rate (0.32 h^{-1}). S_l was the longest in samples stored at 4°C (55.93 h), whereas in samples stored at 25°C it was the shortest (4.28 h). In addition, the tested empirical models were able to accurately *predict Salmonella survival as a function of temperature.*

Significance: Results suggest that contamination by *Salmonella* in yoghurt could pose a significant risk to consumers. The predictive models herein developed could be applied to better support quantitative vulnerability and risk assessment studies, providing more accurate estimates.

T12-03 Behavior of *Staphylococcus aureus* in the Presence of Bacteriocin Producer *Enterococcus faecalis* in Fresh Soft Cheeses

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Introduction: *Staphylococcus aureus* is a pathogen of major concern in foods, especially milk and cheese, due to the production of thermoresistant enterotoxins. Dairy autochthonous microbiota is mainly composed of lactic acid bacteria (LAB) with recognized antagonistic potential over certain pathogenic and spoilage bacteria. Despite this, the impact of bacteriocin-producing LAB over *S. aureus* physiology and virulence in foods is still poorly understood.

Purpose: This study aimed at monitoring *S. aureus* behavior in the presence of bacteriocin-producing *E. faecalis* during the production of a fresh soft cheese.

Technical

Methods: *S. aureus* ATCC 29213 was inoculated (10^3 CFU/ml) alone or in combination with *E. faecalis* 41FL1 (10^6 CFU/ml) in 3L of microfiltered milk, yielding 5 cheeses of approximately 60 g each. The kinetics of bacterial growth, bacteriocin and staphylococcal enterotoxin production was monitored during 21 days.

Results: pH values in cheeses inoculated with *E. faecalis* reached 5.0 from day 0, whereas in cheeses with *S. aureus* alone pH decrease was observed only at day 7. Kinetics of *E. faecalis* growth and bacteriocin production was not altered in the presence of *S. aureus*. In contrast, *S. aureus* population in mixed culture showed a 3-fold decrease when compared to single culture, accompanied by lack of enterotoxin production. These data suggest a remarkable impairment of *S. aureus* virulence in the presence of bacteriocin-producing *E. faecalis* in cheese-mimicking conditions.

Significance: An improved understanding on how *S. aureus* cope with the presence of LAB in cheese can contribute to the development of new prevention strategies against this major foodborne pathogen.

T12-04 Survivability and Biofilm Forming Abilities of *Aspergillus* Species from Powdered Milk

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Introduction: *Aspergillus* molds are the most common types of fungi in the environment. About 16 species of *Aspergillus* molds are known to be dangerous to humans, causing disease and infection.

Purpose: This study investigate the survivability patterns and the capacity of *Lactobacillus rhamnosus GG (LGG)* for inhibition and biofilm control.

Methods: Powdered milk samples from Bodija market, Ibadan were assessed for the presence of *Aspergillus* species using standard methods. Two isolates from powdered milk sample and a laboratory stock culture (*A. niger*) of 10^4 or 10^6 CFU/ml concentrations of each were used for survivability and biofilm development studies over a 5-day period at refrigeration and room temperatures. Inhibition of *Aspergillus* growth and biofilm formation was tested with 10^4 and 10^8 CFU/ml of *LGG*. *Aspergillus* counts and biofilm mass were determined using standard methods and crystal violet binding assay, respectively. Data were analyzed using ANOVA ($P<0.05$).

Results: *A. niger* and *A. flavus* were isolated from powdered milk samples. *Aspergillus* growth was significantly higher in days 3 (8.14 log CFU/5g) and 5 (7.90 CFU/5g) than the day 1 of incubation (6.39 CFU/5g). *A. niger* produced lower fungal load than *A. flavus*. There was a significant difference in mean fungal load with different concentrations of *LGG*. Both *Aspergillus species* yielded the highest fungal load at day 3 of incubation. Similarly, both *Aspergillus spp* produced more fungal load at room temperature than at refrigeration. Biofilm production was significantly higher in day 3 (0.147) than in day 5 (0.106) and 1 of incubation (0.104). Biofilm production was higher at 10^4 CFU (0.119) than at 10^6 (0.118) CFU. There were significant differences in biofilm forming mass between species. Furthermore, a significant reduction in mean biofilm mass was observed in the presence of *LGG*

Significance: *Aspergillus* spp. in this study had biofilm forming abilities. *LGG* is useful in the control *Aspergillus* multiplication and biofilm development.

T12-05 Microbiological Quality and Pathogen Persistence in Probiotic Amended Recycled Sand Bedding in Dairy Barns

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Introduction: Sand bedding in dairy operations offers the advantages of increased milk yield, improved animal welfare and higher hygienic standards compared to other bedding types (e.g., straw). However, waste management represents a key challenge with sand bedding; it cannot be spread onto land or composted. Sand bedding disposal can cost a small dairy farmer over $10, 000 in waste management, hence there is interest in recycling to reduce the financial burden. Although recycling can reduce the amount of sand required to be disposed of there is the risk of pathogen accumulation that can negatively affect herd health.

Purpose: The focus of the current work is to determine the sanitary quality of recycled sand within commercial dairy farms and how pathogen persistence can be reduced in the presence of a *Bacillus* probiotic preparation.

Methods: Sampling and testing of virgin and recycled sand occurred every 6 weeks from May to December. A 35 day microcosm setting was used to determine the impact a *Bacillus* probiotic had on bacteria of interest.

Results: Results to date have established that the microbiological quality of sand deteriorates over time with the accumulation of enteric bacteria. Over a summer season the average values of *E. coli* levels recovered on recycled sand samples taken from the barns was 4.3 log CFU/g that was significantly higher compared to virgin sand (<2 log CFU/g). Recycled sand harbored coliforms (6.3 log CFU/g), *Bacillus* spp (5.3 log CFU/g) and a total aerobic count of 7.8 log CFU/g both of which were significantly higher than fresh sand. Laboratory-based trials have illustrated that inclusion of *Bacillus* probiotic within sand bedding can significantly decrease the persistence of *E.* coli.

Significance: The research is of direct significance to dairy farmers considering implementing recycled sand bedding and in broader terms, by reducing pathogen prevalence as part of a One Health approach.

T12-06 Transforming Raw Milk into Safe Milk Using Electron Beam Processing

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❖ ***Developing Scientist Competitor***

Introduction: Raw milk consumption is increasing, and illnesses associated with raw milk are also increasing. Raw milk, though sterile in the udder, is a host to a vast microflora of bacteria, including pathogens.

Purpose: We hypothesize that electron beam (eBeam) processing, a non-thermal food processing technology, is suitable for eliminating microbial pathogens in raw milk without detrimentally affecting nutrient content or sensory attributes.

Methods: Bioburden reduction analysis was conducted, using non-selective agar to enumerate raw and eBeam pasteurized milk irradiated at 1.0k Gy and 2.0k Gy. Pathogen reduction analysis was accomplished by inoculating raw milk with *C. burnetii* and overnight cultures of *S. aureus*, *E. coli* O157:H7, *C. jejuni*, and *L. monocytogenes* to determine the D-10 values of each pathogen. Nutritional analysis targeting calcium and vitamins B_2 and $B_{12}$was performed, and GC-olfactometry was used to perform aroma analysis.

Results: The *D*-10 values for the pathogenic organisms was found to range between 74.87 Gy to 156.26 Gy, with *L. monocytogenes* being the most resistant pathogen. Processing raw milk at 1.0k Gy achieved between 4- and 5-log reduction of the natural bioburden. With the exception of vitamin B_2, there was no significant difference in the nutrient content and aroma profiles of the raw milk and eBeam milk processed at 1.0 and 2.0 kGy.

Significance: Current heat pasteurization standards for milk are designed to achieve a 6-log reduction of *C. burnetii*, and based upon the *D*-10 analysis of the target pathogens and the empirical data from the bioburden reduction studies, it appears that 2.0 kGy is effective at pasteurizing raw milk without affecting major milk nutrients or aroma profiles. Overall, these results point to the value of eBeam technology to meet the needs of consumer demand for raw milk while assuring the microbiological and public health safety of such non-thermally pasteurized milk.

T12-07 Survival of Hepatitis A Virus and Aichi Virus in Cranberry-based Juices at 4°C

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Introduction: Foodborne viral illness continues to be a cause of concern globally, with reported annual outbreaks of hepatitis A virus (HAV) linked to fruits and juices. Aichi (AiV) virus is an emerging foodborne viral pathogen with limited data on its epidemiology. Both HAV and AiV are resistant to low pH and can survive under adverse environmental conditions that leads to their ease of transmission and outbreaks.

Purpose: The objective of this study was to evaluate the survival of HAV and AiV in cranberry-based juices (27% cranberry juice cocktail, CJ and a mixed juice with cranberry, MJ) over 0, 1, 2, 3, 4, 5, 6, 7, 14, and 21 days at refrigeration (4°C).

Methods: Two commercially available cranberry-based juices and phosphate buffered saline (PBS, control) were inoculated with each virus (final titer of 6 log PFU/ml) and stored at refrigeration over 21 days. At each time interval, the inoculated juices were serially diluted in cell-culture media and standard plaque assays with confluent host cells in 6-well plates were used to determine viral infectivity. Each experiment was carried out in duplicate and replicated thrice and data were statistically analyzed.

Results: Reductions ranging from 0.72±0.06 to 2.3±0.18 log PFU/ml and 0.63±0.02 to 1.84±0.14 log PFU/ml were obtained over 21 days for AiV with MJ and CJ, respectively. Reductions ranging from 0.67±0.03 to 1.09±0.1 and 0.93±0.27 to 1.49±0.18 log PFU/ml were obtained for HAV over 21 days at refrigeration in MJ and CJ, respectively. Both AiV and HAV were found to survive storage at refrigeration. In the PBS control, AiV showed merely 0.59 log PFU/ml reduction and HAV showed insignificant 0.04 log PFU/ml reduction ($P\geq0.05$) after 21 days at refrigeration.

Significance: The data obtained from this study will help to determine the risk of HAV and AiV transmission from cranberry-based juices and provide data for risk-modeling and risk assessment studies.

Poster Abstracts

P1-01 Assessing Food Safety Risks On-farm through Environmental Monitoring

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✦ ***Undergraduate Student Award Competitor***

Introduction: Environmental monitoring is an effective tool to provide risk indicators within food production and can be used to assess and implement best management practices. Since produce is a leading source of foodborne illness, there is a strong need to provide extension programming to develop tools to adopt these practices.

Purpose: The purpose of this study is to use environmental monitoring tools to identify risks areas on-farm. The results across two growing seasons (2014 and 2015) help to develop outreach tools for improved management practices through extension activities.

Methods: Weekly, water samples were collected from two locations on the Connecticut River and tested with the Quanti-Tray 2000 MPN system. Environmental samples were collected from the UMass Research farm in Deerfield, MA and plated within 24 hours of collection on 3M APC and *E. coli*/Coliform Petrifilm. Statistical analysis (*t*-test) was conducted to determine differences between harvest years.

Results: Water samples gave a geometric mean of 62.37 MPN/100 ml generic *E. coli* across two years. This data complies with the geometric mean of the FDA Produce Rule (≤126 CFU generic *E. coli*) but would not comply with the statistical threshold (<410 CFU generic *E. coli*) since select samples exceeded this number. On-farm, drains averaged 7 logs in coliform counts across two seasons. The produce-washer, a food-contact surface, gave coliform counts as high as 7 logs on various surfaces of the unit. Other areas of the room, such as the floors, which are not food-contact surfaces but high risk, averaged coliform counts of 6 logs.

Significance: This data demonstrates microbial risk on-farm, leading to the need for extension education. The results identify focus areas for additional research and extension programs to improve management practices for on-farm food safety.

P1-02 Visible "Soil" as an Indicator of Bacterial Concentration on Farmworkers' Hands

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Introduction: Effective hand hygiene techniques for farmworkers are necessary to reduce hand to produce microbial contamination and produce-associated illness. Anecdotally, visible "soil" on worker hands has been assumed to be an indicator of hand contamination, including microbial contamination.

Purpose: To address this assumption, the goal of this project was to determine if, on farmworker hands, visible "soil" on farmworker hands was associated with microbial load.

Methods: A total of 181 farmworkers were asked to wash their hands to standardize hand microbial loads. Workers were then asked to harvest tomatoes without gloves for 30 minutes, practice hand hygiene (e.g., hand wash, hand sanitizers) and then rinse their hands in 0.1% peptone solution. The solutions were analyzed for $Absorbance_{600nm}$ (turbidity) and microbial load (*Enterococcus* spp., coliforms and *E. coli*). Hands were photographed after rinsing and assigned a "Hand Score" based on visible soil on the palm, soil on the finger pads, soil under the fingernails, multicolored soil, and total soil by two independent reviewers.

Results: Hands ranged in $Absorbance_{600nm}$ (0.175 ± 0.19 SD), Hand Score (3.9 ± 1.8 SD), log *E. coli* CFU/hand (1.40 ± 0.73 SD), log *Enterococcus* (3.84 ± 1.58 SD), and log coliforms (2.61 ± 1.59 SD). Using Spearman's correlation tests, Absorbance and Hand Score were significantly correlated (rho=0.540, $P<0.001$). In contrast, Hand Score and concentrations of *E. coli* (rho=0.025, $P=0.826$) and coliforms (rho=-0.089, $P=0.440$) were not significantly correlated. Hand Score and concentrations of *Enterococcus* (rho=0.273, $P=0.015$) were significantly weakly correlated.

Significance: Our results suggest that while visible hand "soil" is a good proxy for hand rinse turbidity, visible "soil" is not an indicator of microbial load for all microorganisms on farmworker hands. Thus, farmworkers or managers cannot depend on visual "cleanliness" alone to assess their hands' microbiological "cleanliness;" performing a quality hand hygiene intervention is recommended.

P1-03 Microbial Loads of Fresh Produce and Packing Equipment Surfaces Collected Near the U.S. and Mexico Border are Associated in Packing Facilities

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❖ ***Developing Scientist Competitor***

Introduction: Several produce-associated outbreaks have been linked to the packing facility. Equipment surfaces may be an important source of contamination.

Purpose: The goal was to assess whether the microbial load of packing facility surfaces are associated with the microbial load of produce.

Methods: From November 2000 to December 2003, 487 matched produce (14 types) and equipment surfaces (6 production steps) were sampled from 8 packing facilities near the U.S. and Mexico border and enumerated for aerobic plate counts (APC), generic *E. coli*, enterococci, and coliforms. Bivariate correlations were assessed by Spearman's rho and adjusted associations were assessed by multilevel mixed linear regression models.

Results: In general, the microbial loads on produce and equipment surfaces changed across production steps but did not consistently trend in any one direction. Equipment surface and produce microbial loads were correlated, but correlations varied from none to high depending on the equipment surface. For example, significant correlations ($P<0.01$) included: APC (rho=0.386) and enterococci (rho =0.562) with the harvest bin, *E. coli* (rho =0.372) and enterococci (rho =0.355) with the merry go round, enterococci (rho =0.679) with rinse cycle equipment, APC (rho =0.542) with the conveyer belt, and for all indicators with the packing box (rho=0.310-0.657). To identify and compare equipment surfaces at elevated risk for contaminating produce, a model was constructed that controlled for crop type, produce-swab group, and sampling location. There were significant positive associa-

tions between the log concentration of enterococci on produce and the harvest bin (beta=0.259, *P*<0.01) and the rinse cycle (beta=0.010, *P*=0.01), and between the log concentration of all indicators on produce and the packing box (beta = 0.155-0.500, all *P*<0.01).

Significance: Statistically significant associations between microbial loads on packing facility surfaces and fresh produce confirm the importance of packing facility sanitation to protect produce quality and safety.

P1-04 Survival of STEC and *Salmonella* Serotypes in Florida Animal Feces

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❖ ***Developing Scientist Competitor***

Introduction: Domestic and wild animal intrusion represents a significant route of pathogen contamination during produce production; limited data have been published regarding the survival capabilities of pathogens in animal feces.

Purpose: The purpose of this study is to *compare the* survival of Shiga toxigenic *Escherichia coli* (STEC), *Salmonella*, total aerobic count (APC), and generic *E. coli* in cattle and different wild animal feces from Florida.

Methods: Rifampicin resistant 5-strain cocktails of STEC and *Salmonella* were inoculated into domestic cattle and wild animal feces (10^5 to 10^6 CFU/g; deer, wild pig, raccoon, and waterfowl). Fecal samples were stored at room temperature. Populations were enumerated for up to 1 year by spread plating onto TSAR. If no colonies were detected samples were enriched. Control samples were enumerated on TSA and CHROMagar ECC to monitor APC and generic *E. coli* concentrations. Models for comparing STEC, *Salmonella* and generic *E. coli* populations were developed.

Results: Over the 364 day storage, STEC and *Salmonella* populations declined by 2.4 and 0.8 log CFU/g in the cattle feces, and 2.1 and 1.6 in the deer feces, respectively. STEC populations rapidly declined during the first 28 days in pig, waterfowl, and raccoon feces, to ≤1.7 log CFU/g. Similarly, *Salmonella* populations in pig, waterfowl, and raccoon feces, declined to ≤2.6 log CFU/g over the first 56 days. No subsequent significant population changes (*P*>0.05) occurred during the remaining storage of pig, waterfowl, and raccoon feces after day 84 for STEC and day 112 for *Salmonella.* The rate of generic *E. coli* decline corresponded to pathogen decline in all fecal types. The calculated predictive power (R^2) of the models relating pathogen declines to generic *E. coli* in each fecal type ranged from 0.4362 to 0.9774.

Significance: Determining appropriate risk mitigation strategies following animal intrusion can improved with a better understanding of pathogen survival in animal feces.

P1-05 Role of Bird Droppings in Microbial Dispersal of Generic *E. coli* and *Salmonella* in Field-grown Tomatoes in Florida

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Introduction: A range of actions may be taken when bird droppings are observed on tomatoes: from not harvesting the specifically contaminated fruits to removing the plant with visible bird droppings and the adjacent plants.

Purpose: The aim of this study was to determine the microbial dispersal due to bird droppings on tomato plants in the field.

Methods: Four experimental tomato fields, planted and maintained at typical commercial standards, were visited at least twice while fruit were mature (10 visits), and surveyed for contamination with bird droppings. Mature fruit with visible bird droppings, fruit not visually contaminated but within 30 cm of the contamination, fruit on the same plant but more than 30 cm from the contamination, and fruit on the adjacent plants were evaluated for aerobic plate count (APC), coliforms, generic *E. coli*, and *Salmonella*. Control fruit were collected from a plant ca. 10 feet away that had no visible bird droppings. Significant differences were determined using the Student's *t*-Test with Bonferroni correction.

Results: *Salmonella* was isolated from 1/356 samples, collected from fruit on a plant adjacent to a plant contaminated with bird droppings. Populations of coliforms and APC (ca. 2.5 and 7.0 log MPN/tomato, respectively) were not significantly different between contaminated and control fruit (*P*=0.35 and *P*=0.07, respectively). Generic *E. coli* was higher (*P*=0.008) on contaminated fruit (0.5 ± 0.9 log MPN/tomato) than control fruit (0.1 ± 0.2 log MPN/tomato). Generic *E. coli* on tomatoes within 30 cm of contaminated fruit (0.1 ± 0.3 log MPN/tomato) was not significantly different than the controls (*P*=0.37).

Significance: Prevalence of *Salmonella* on Florida tomatoes was <1%. Enumeration of samples for generic *E. coli* suggest that these microbial populations are not dispersed in high numbers by bird droppings beyond visually contaminated tomatoes.

P1-06 Effects of Distance on Risk Associated with Wildlife Encroachment in Field-grown Leafy Greens

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❖ ***Developing Scientist Competitor***

Introduction: Previous research has generally supported the 5-ft no-harvest zone currently used as a means of managing produce safety risks concerning wildlife fecal contamination.

Purpose: Here studies evaluated generic *E. coli* and fecal coliforms (FC) as indicators of contamination associated with wildlife fecal material on the surrounding plants and soil within a measured buffer zone.

Methods: Composite leaf (25 g) and soil (100 g) samples were collected from fields in Delaware and California from the summer 2015 to spring 2016. Sampling occurred around the buffer circumference randomly starting at 10 ft from the identified fecal contamination, and moving inward at 5 distances. A total of 5 pooled leafy green and 5 pooled soil samples were analyzed for generic *E. coli* and FC at each fecal event.

Results: In Delaware, no generic *E. coli* was detected except at 7ft (3.2 x10^2 CFU/sample) in soil (1/15). Fecal coliforms were detected in lettuce (6/20) and soil (9/19), ranging from (3.5 x 10^2 to 1.7 x 10^4 CFU/sample). In California, generic *E. coli* was detected in soil (2/15) samples only at 0 ft (3.5 x 10^5 CFU/sample) and 1 ft (3.4 x 10^5 CFU/sample) from fecal material. Fecal coliforms were detected in lettuce (3/13) and soil (5/15), ranging from (9.5 x 10^3 to 1.1 x 10^6 CFU/sample). In both regions, generic *E. coli* was undetectable on lettuce and spinach samples from all 5 distances (0/29) and recovery of FC was too sporadic to determine a significant effect by distance.

Significance: At each fecal event, generic *E. coli* and FC were enumerated in surrounding leafy greens and soil within and outside of the 5-ft no-harvest buffer zone, suggesting that harvesting leafy green in close proximity to fecal contamination may pose a potential food safety hazard.

Poster

P1-07 Evaluation of Bioaerosol Dispersal and Deposition Relative to Setback Distances between Manure Sources and Fresh Produce Crops

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Introduction: Animal rearing and manure handling facilities are considered possible sources of human pathogens that may aerosolize and deposit onto nearby fresh produce, thereby increasing foodborne illness risk potential of the impacted commodities. Highly variable meteorological, landscape, and farm conditions make prediction of set-back buffer distances from farm animal and manure handling sources exceptionally challenging.

Purpose: The objective was to evaluate the influence of a range of on-site conditions on bioaerosol concentrations, transport, and deposition, and then use dispersion models to determine set-back distances between fresh produce operations and farm manure sources of fecal bacteria.

Methods: Several field experiments were conducted during typical animal rearing/manure handling operations. Bioaerosols from poultry house ventilation exhaust and during poultry litter and dairy manure handling and composting operations were collected 3-182 m downwind using Anderson impactors, SpinCon cyclonic air samplers, and open-face filters. Deposition was determined using tomato, lettuce, spinach, and basil plants and sterile water basins. Total aerobic bacteria, fecal coliform and *E. coli* concentrations were determined by conventional plating and MPN procedures (*n*=300 samples). Emission rates were derived and used to model airborne concentrations and deposition at various distances downwind of the sources under a range of meteorological and landscape conditions.

Results: Source strength, manure handling and moisture content, along with meteorological conditions, downwind distance, and vegetative buffer belts impacted the concentrations of viable airborne target bacteria and their downwind deposition onto plants and water surfaces. The complexity of multiple interacting factors makes atmospheric dispersion and deposition modeling essential methods to predict the likelihood of produce contamination events and the most conducive conditions.

Significance: Determining reasonable setback distances from suspected sources of zoonotic pathogens in order to protect nearby fresh produce crops from contamination via bioaerosol deposition will benefit from consideration of site-specific factors and use of atmospheric dispersion models.

P1-08 Survival of Generic *E. coli* and Naturally Occurring *Listeria* spp. in Manure-amended in Loamy and Sandy Soils in the Northeastern United States

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Introduction: Enterohemorrhagic *Escherichia coli* and *Listeria monocytogenes* can contaminate leafy greens through inappropriately managed raw manure applied to soils. Current FDA regulations call for a 90-120 day interval between application of manure and harvest of crops grown in manure-amended soils.

Purpose: To determine if addition of dairy-manure solids (DS) affects persistence of *E. coli* and *Listeria* spp. in soils.

Methods: A three-strain inoculum (TVS353,TVS354, and TVS355) of rifampicin-resistant *E. coli* (*rEc*) (6 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). Manure was either tilled into soil or spread on the surface, and each plot was seeded with spinach ('Space' variety). Survival of inoculated *rEc*, naturally present *E. coli* (*nEC*) and *Listeria* spp. in DS in untilled and tilled plots were quantified over 80 days-post-inoculation (dpi) by colony count or MPN.

Results: By 80 dpi, in untilled plots, *rEc* populations declined by 2.6 – 3.9 and 2.4 – 2.7 log CFU/g in NM and DS plots, respectively. In tilled plots, *rEc* populations declined by 0.9 – 1.1 log CFU/g and1.1 – 1.2 log CFU/g in NM and DS plots, respectively, at 80 dpi. *Listeria* spp. populations declined by 0.62 - 1.3 log CFU/g in tilled DS plots, and by 1.2- 2.6 log CFU/g in untilled NM by 80 dpi. In tilled DS plots, *nEC* populations declined by 0.6 – 0.7 log CFU/g by 28 dpi, compared to declines of *rEC* of 0.2 – 0.3 log CFU/g by 28 dpi. BOX-PCR-typing revealed that 72.5% of the *rEc* isolates recovered at 65 dpi from the rhizosphere, were strain TVS355.

Significance: Tilling, with or without DS, slowed declines of *rEc* populations in soils. However, the presence of DS slowed the decline of *Listeria* spp. in tilled soils. Population declines of *E. coli* and *Listeria* spp. were not affected by soil type.

P1-09 Survival of *Clostridium difficile* in Finished Dairy Compost under Controlled Conditions

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Introduction: *Clostridium difficile*, the most common nosocomial human pathogen, has also been diagnosed as a community associated pathogen. Fecal matter of contaminated animals or humans is the source of *C. difficile*.

Purpose: To assess the survival of vegetative cells and spores of *C. difficile* in finished dairy compost by simulating pre-harvest contaminations of agro-production.

Methods: Both autoclaved and unautoclaved finished dairy composts at 20, 30, and 40% moisture levels, were inoculated with *C. difficile,* grown in brain heart infusion (BHI) broth supplemented with yeast extract and L-cysteine for 12 h, at concentrations of 5-6 log CFU/g of vegetative cells and spores and held at room temperature for up to 120 days. The surviving vegetative cells and spores in duplicate compost samples were enumerated on BHI agar and BHI agar supplemented with 0.1% sodium taurocholate, respectively, at selected time intervals. The experiment was repeated three times for each moisture level. Variance of cell and spore counts were analyzed using Tukey's multiple-comparison procedure of the Statistical Analysis System at a *P*-value of 0.05.

Results: During the entire study, the change of moisture content of both composts at each set moisture level was not significantly different. The vegetative cell counts rapidly declined during storage for both types of compost and after 24 h, the declines were 4-5 log CFU/g at 20% moisture, and ca.1.5-2.5 log CFU/g for 30 and 40% moisture levels. However, vegetative cells were detectable for up to 30, 120, and 60 days, respectively, at 40, 30, and 20% moisture levels in both types of compost. In contrast, the spore counts were not significantly different for 120 days in both types of compost at all three moisture levels.

Significance: The extended survival of *C. difficile* spores in compost suggests the contaminated compost/manure-amended soil could serve as a potential source of *C. difficile*, that contaminates fresh produce, water or field workers on farm.

P1-10 Influence of Mulching on Foodborne Pathogen Persistence in Soil

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Poster

Introduction: Mulching is a common practice to control weed growth and retain soil moisture during crop production. The effect of mulching on the persistence of human pathogens in soil is unknown. Enhanced survival of pathogens under mulch may increase the risk of contamination of produce.

Purpose: The purpose of this study was to determine the fate of foodborne pathogens in soils covered with polyethylene plastic, biodegradable plastic, paper or straw mulches, or left uncovered (bare ground).

Methods: The surface 2 cm of soil in troughs (60 x 17 x 10 cm) were inoculated with a ~6 log CFU/ml cocktail of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* Newport. Soil troughs were covered with one of polyethylene plastic, biodegradable plastic, paper or straw mulch, or left uncovered as a bare ground control (4 replicates each), and held in a growth chamber maintained at day and night cycles of 17°C for 9 h and 30°C for 15 h, respectively. Soil samples (1 g) were collected periodically over a two week period during the first trial, and a three week period during the second trial. Pathogens in soil were enumerated by spread plating on selective agars. Significant differences among mulches and pathogens were determined by one-way analysis of variance (ANOVA) and Tukey's test (significance level 0.05).

Results: Populations of pathogens in soils decreased significantly over time in both trials (P<0.01). Mulch had a significant effect on the survival of pathogens over time compared to bare ground (P<0.01), with the greatest pathogen survival under straw mulch. In both trials, *L. monocytogenes* persisted at significantly higher populations than *E. coli* O157:H7 and *S. enterica* Newport (P<0.01), and populations of *E. coli* O157:H7 declined the most rapidly (P<0.01).

Significance: Enteric pathogens persisted longer in soil covered with mulch compared to bare ground, with straw exhibiting the most significant effect.

P1-11 The Effect of Gastric Acidity on *Escherichia coli* Isolates Recovered from Poultry Litter-amended Soils

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Introduction: Pathogenic *E. coli* can contaminate fruit and vegetable commodities, and may be introduced to soils through biological soil amendments. It is unknown how survival in manure-amended soils affects the tolerance of *E. coli* to low pH environments. Gastric acidity in humans is one of the barriers pathogenic *E. coli* need to overcome to cause infection.

Purpose: To determine if *E. coli* recovered from poultry litter (PL)-amended soils have greater tolerance to gastric acidity than laboratory strains.

Methods: Several field studies conducted between 2011 and 2014 examined the survival of a three-strain inoculum (TVS353, TVS354, and TVS355) of rifampicin-resistant *E. coli* (*rEc*) laboratory isolates (LI) in PL-amended soils in Maryland and Pennsylvania. Samples of PL-amended soils were taken for up to 341 days post inoculation (dpi) and analyzed for *rEc* populations. Colonies of field isolates (FI) were identified by BOX-PCR-typing. FI of TVS353 and TVS354 were recovered at 120 dpi and 150 dpi, respectively, while two FIs of TVS355 were recovered at 150 dpi and another recovered at 341 dpi. FIs and LIs of each strain were exposed to synthetic gastric fluid (SGF, pH 2.3) for 0, 4 and 6h and changes in populations (log CFU/ml) were statistically compared by Student's- *t*-Test.

Results: All LI and FI *E. coli* populations declined by 0.8 – 2.6 log CFU/ml and by 2.0 – 3.6 log CFU/ml after 4 and 6 h, respectively, of exposure to SGF. There were no significant (P > 0.05) differences between LI and FI isolates of either TVS354 or TVS355. However, at 4 h, for TVS353, FI population declines (2.6 log CFU/ml) were significantly greater than those of LI (1.5 log CFU/ml).

Significance: Extended survival of *E. coli* in PL-amended soils did not affect the acid tolerance of *E. coli* strains compared to laboratory strains; however, extended survival in manure-amended soils may induce other phenotypic changes in *E. coli*.

P1-12 Selection of Indigenous Indicator Microorganisms for Validating Desiccation-adapted *Salmonella* Reduction in Physically Heat-treated Poultry Litter

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❖ ***Developing Scientist Competitor***

Introduction: Physical heat treatment is commonly used to eliminate human pathogens, such as *Salmonella*, in poultry litter as organic fertilizer. However, it is not feasible in poultry litter processing facility to validate thermal processes with pathogenic bacteria.

Purpose: The objective of this study was to compare the thermal inactivation of desiccation-adapted *Salmonella* with that of indigenous indicator microorganisms in poultry litter.

Methods: Aged broiler litter and composted turkey litter with 20, 30, 40, and 50% moisture contents were inoculated with desiccation-adapted *Salmonella* Senftenberg (ca. 10^7 log CFU/g) and then heat-treated at 75 and 85°C for 3 h. The surviving populations of *Salmonella* Senftenberg, indigenous enterococci, and total aerobic bacteria were enumerated.

Results: Compared to total aerobic bacteria, there were better correlations between mean log reductions of desiccation-adapted *Salmonella* Senftenberg and indigenous enterococci in broiler litter samples with 20, 30, 40, and 50% moisture contents at 75°C (R^2>0.91), and 20, 30, and 40% moisture contents at 85°C (R^2>0.87). Mean log reductions of *S.* Senftenberg were better correlated with those of indigenous enterococci in turkey litter samples with 20, 30, 40, and 50% moisture contents at 75°C (R^2>0.88), and 20 and 30% moisture contents at 85°C (R^2=0.83) than those of total aerobic bacteria, which had a better correlation in turkey litter sample with 40% (R^2=0.98) moisture content at 85°C.

Significance: Our results demonstrated that indigenous enterococci may be used to validate the thermal processing of poultry litter as it mimics the survival behavior of *Salmonella* during heat treatment.

P1-13 Diversity and Dynamics of *Salmonella enterica* spp. in Irrigation Water and Poultry Litter Amended Fields on the Eastern Shore of Virginia

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Introduction: Recurring salmonellosis outbreaks associated with vegetables in Delmarva region raised concerns about the contamination risks of foodborne pathogens in the agricultural environment.

Purpose: A two-year study was performed to investigate the diversity and dynamics of *Salmonella enterica* spp. in irrigation water and poultry litter amended fields on the eastern shore of Virginia.

Methods: Irrigation pond water and well water were sampled weekly from four vegetable farms (farm A-D) in 2014 and 2015. Soil samples were collected monthly after fertilization with chicken manure from 10 farms in 2014, and another 14 farms in 2015. The most probable number (MPN) method was used to quantify *Salmonella* spp. in the samples. Presumptive *Salmonella* colonies were confirmed by the cross-streaking method. Molecular serotyping was carried out to determine *Salmonella* serovars.

Results: The observed prevalence of *Salmonella* in pond irrigation water was approximately the same in 2014 (19.9%) and 2015 (19.4%), while the average concentration in the water increased from 0.49±1.66 MPN/L in 2014 to 0.82±5.14 MPN/L in 2015. All wells were *Salmonella* positive except from farm B at certain time points, especially in farm D. The prevalence (<4%) of *Salmonella* in Well water was significantly lower than that for Pond water (P<0.01), and had an average level value of 0.03±0.15 MPN/L in 2014 and 0.05±0.36 MPN/L in 2015. *Salmonella* was found to be able to survive up to four months in poultry litter amended soils from the tested farms in 2014, and up to six months in 2015. There were spatial and temporal differences for *Salmonella* serovar diversity in water and soil samples.

Significance: This research examined the dynamics of *Salmonella* spp. in relationship to irrigation water sources and poultry litter used for vegetable production, and provides useful information for food safety risk assessment.

P1-14 Survival of *Salmonella enterica* spp. in Poultry Litter-amended Fields and Inoculated Soil

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Introduction: *Salmonella enterica* is the most frequently encountered bacterial pathogen associated with foodborne illness in the United States.

Purpose: To investigate the survival of *Salmonella* spp. in fields amended with poultry litter, and in artificially inoculated soil in a growth chamber.

Methods: In field experiments, naturally contaminated poultry litter with *Salmonella* (700 MPN/kg) was collected and amended into soil to emulate manure fertilization at four levels of 0 kg (control), 5 kg, 25 kg, and 100 kg per plot (45 m^2). Soil samples were tested monthly for *Salmonella* contamination by the MPN method for nine months. In a laboratory experiment, a *Salmonella* cocktail inoculum of five serovars was mixed into soils collected from tomato fields to reach initial concentrations of 2, 4 and 6 log CFU/g. *Salmonella* populations after treatment were determined daily in the first week, and weekly for next three months. A randomized block design with four replications was performed in both field and laboratory experiments.

Results: In field experiments, *Salmonella* was isolated from plots that had received 25 kg of poultry litter in month 1 with an average population density of 12 MPN/kg (95% CI: 5.4 to 25 MPN/kg). Plots treated with 100 kg poultry litter were found positive for *Salmonella* four months after soil amendment with initial concentration of 14 MPN/kg (95% CI: 6.8 to 28 MPN/kg). No *Salmonella* was isolated from the 5 kg treatment or control plots during the study. In laboratory experiments, *Salmonella* populations decreased in inoculated soils over the testing period. The pathogen was no longer detected after 5, 7, and 9 weeks after inoculation with initial concentration of 2, 4 and 6 log CFU/g, respectively.

Significance: Data derived from the study can be used in risk assessments to determine the public health implications of using raw manure as a soil amendment.

P1-15 Transport of Pathogens in Runoff from Soil Amended with Manures

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Introduction: Microbial contamination of fresh produce can occur through various pathways, including irrigation water, flooding, Ag-sprays, and runoff. Manure application to cropland may produce pathogens in runoff water; raising concerns regarding potential for contaminating fresh produce with enteric pathogens.

Purpose: This study examined bacterial transport in runoff from manure-amended soils subjected to rainfall simulation events.

Methods: A rainfall simulation study assessed *Salmonella* and *E. coli* in surface runoff from Othello silt-loam soil packed in stainless-steel boxes (0.00002 ha) amended with dairy slurry (DS), liquid swine manure (LSM), poultry litter (PL), and composted PL, (surface-applied, 3 mt/ha), and one treatment of incorporated poultry litter. Four rainfall-simulation events (7 cm/h for 40 minutes each) were conducted at weekly intervals. *Salmonella* and *E. coli* were enumerated from collected runoff water.

Results: Initial *Salmonella* and *E. coli* populations in DS-amended boxes were 4.60 and 6.61 log CFU/g, respectively, whereas in LSM the populations were 4.08 and 5.08 log CFU/ml, respectively. *Salmonella* was not detected in PL or PL-compost runoff; *E. coli* was detected in runoff from PL-compost-amended soil (0.40-1.15 log CFU/ml), and in significantly (P<0.01) greater amounts in runoff from broadcast than incorporated PL-amended soil after rainfall events 2 and 4. *Salmonella* and *E. coli* populations detected in runoff water from dairy slurry- and liquid swine manure-amended soil during the first rainfall simulation event ranged from 2.0-3.2 log CFU/ml and 4.0-4.3 log CFU/ml, respectively. *E. coli* populations in both manure slurries were comparable (2.6 log CFU/ml); *Salmonella* was not detected in runoff water from the second rainfall simulation event.

Significance: This study shows that manure type influences pathogen concentrations and persistence in surface runoff water collected immediately after manure application and rainfall in comparison to concentrations several days later. This indicates that soil retention or bacterial die-off could have contributed to pathogen reduction in the interim.

P1-16 Rainfall Promotes Growth of Fecal Coliforms in Soil and on Leafy Greens during Production in the Mid-Atlantic Region of the United States

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Introduction: Previous field studies have reported increases in recovery of fecal coliforms (FC) from produce after a rain event. It is unclear whether this is a consistent occurrence, and how long increased population levels persist on crops. Enhanced bacterial growth following rainfall signals a possible food safety risk and would have significant implications for harvesting practices.

Purpose: This study evaluated the effect of rainfall events on naturally-occurring FC populations in soils and on leafy greens being cultivated on commercial farms.

Methods: For three days following significant rainfall (>1.3 cm rainfall within 24 h), leafy green and soil samples (5 replicates each) were collected from farms in Maryland, Delaware and New Jersey. *Escherichia coli* and FC were enumerated by direct plate counts on TBX agar. Samples were also collected before rainfall to determine baseline populations of these microorganisms. Data were collected surrounding rainfall events occurring during the spring and fall growing seasons of 2015.

Results: On central Maryland and Maryland eastern shore farms, a significant increase in recovery of FC was observed in both soil and on leafy greens after rainfall in fall (P<0.05) but not spring. A similar increase in recovery occurred in fall for farms in central and southern New Jersey (P<0.05).

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In Delaware, FC populations in soil remained constant before and after rain, but an increase in FC levels in leafy greens samples was observed in spring, although the effect was not significant. In all regions, recovery of *E. coli* was too low or sporadic to determine a significant effect of rain.

Significance: Following rain, FC populations surged in soil and on leafy greens during production, especially in fall. Levels returned to baseline within two days. Harvesting delays following rain may reduce any potential microbial food safety risk posed by rainfall for leafy greens.

P1-17 Response of Cucumber and Tomato Microbiomes to Rainfall

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❖ ***Developing Scientist Competitor***

Introduction: Cucumber and tomato are frequently consumed raw and have been implicated in several foodborne disease outbreaks. Research indicates that numbers of fecal indicator bacteria on crops spike immediately following a rainstorm, potentially representing an increased food safety risk.

Purpose: We profiled the surface bacterial communities of cucumber fruit, tomato fruit, and tomato leaves in the days surrounding two rainstorms to investigate the influence of rain on the microbiome of these high risk crops.

Methods: Cucumber fruit (*n*=24) and tomato leaf (*n*=35) and fruit (*n*=35) samples were collected from a Maryland organic farm on 5 dates surrounding 2 rain events (12.45 mm and 3.56 mm). DNA was extracted from surface washes, and 16S rRNA gene sequencing was performed using Illumina MiSeq v3. Data was quality filtered and analyzed using QIIME v. 1.8.

Results: At a sampling depth of 15,540 sequences per sample, approximately 1,055 OTUs (97% similarity) were identified for each sample. A significant influence of collection date on microbial community composition was observed on cucumbers (R^2=0.225, *P*=0.002), with 4 days pre-rain and 1 day post-rain samples hosting significantly different communities (*P*=0.002); 4 day post-rain samples were indistinguishable from the two other time points. A weaker, but still significant, effect of sampling date was observed on tomato fruit communities (R^2=0.12, *P*=0.003) but not tomato leaf-associated communities (R^2=0.06, *P*=0.43). Both cucumber and tomato fruit-associated communities showed a temporary increase in Betaproteobacteria immediately following rain, which was partially reversed by 4 days post-rain.

Significance: This study demonstrated a shift in the structure of cucumber and tomato fruit surface-associated bacterial communities following a rain event. The susceptibility of plant hosts to pathogen colonization could be enhanced by these weather-induced shifts in microbial species dominance, and this shift may be more dramatic on fruits.

P1-18 *Salmonella* Transport through Irrigation Systems and the Risk of Fresh Produce Contamination on Farms in Southern Georgia

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Introduction: Fresh produce can become contaminated through numerous pathways from farm to fork. Contaminated irrigation water has been cited as a source of outbreaks linked to fresh produce. Surface water ponds are used for irrigation on many farms in southern Georgia even though *Salmonella* has regularly been detected in surface water in the region in low concentrations. *Salmonella* can survive in non-host environments, which potentially poses a hazard to produce safety.

Purpose: The purpose of this study was to evaluate the prevalence of *Salmonella* in surface water ponds, irrigation systems, and on fresh produce crops at harvest on farms in southern Georgia, in order to assess the risk for pre-harvest contamination of produce irrigated with surface water.

Methods: Ninety-four water samples (2 L) from irrigation sources (surface water ponds and wells) and associated distribution systems (drip irrigation, sprinkler, and pivot), and 65 standardized units of produce were collected. *Salmonella* concentrations were enumerated using a most probable number (MPN) method. Presumptive positives were confirmed using PCR and isolates were analyzed with pulsed-field gel electrophoresis (PFGE).

Results: *Salmonella* was detected in 29% of irrigation ponds and systems throughout the study, with positive samples ranging in concentration from 0.055 MPN/100 ml to 1.8 MPN/100 ml. Two out of 65 produce samples (cantaloupe and cucumber) were positive for *Salmonella*, but concentrations were low (1.1 MPN/sample). PFGE analysis indicated 26 distinct pulsotypes present in the samples and that strains found in irrigation ponds were also found in irrigation systems. Strains found on crops were also detected in the irrigation systems, indicating that *Salmonella* in water from irrigation systems could be a source of contamination of produce.

Significance: This study has shown that pre-harvest contamination of produce with *Salmonella* occurs on farms that irrigate with surface water contaminated with *Salmonella*.

P1-19 Assessment of Generic *E. coli* in Surface Irrigation Water Sources and Fruit in Selected Michigan Blueberry Farms

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Introduction: Fresh produce that is consumed raw can be a source of microbial pathogens that can cause human illness. This is particularly true for produce that is irrigated with untreated surface water at times close to harvest. Michigan has a large number of blueberry farms, and many of these farms irrigate their crops using untreated water drawn from irrigation ponds.

Purpose: The purpose of this research was to quantify generic *E. coli* levels in repeated samples obtained from irrigation water sources at six blueberry farms in Michigan.

Methods: The farms for this research were selected based on their use of surface water as irrigation water sources, and on their susceptibility to runoff from their surroundings. Samples of water and blueberries were collected from late June until mid-September in 2015. Water samples were obtained twice weekly and blueberry samples once weekly during the course of the experiment. The Colilert and Quanti-Tray/2000 method was used for quantifying generic *E. coli* levels.

Results: Among the water samples (*n*=111), 94% contained detectable generic *E. coli*, and 19% of samples had generic *E. coli* levels greater than 126 MPN/100 ml. The maximum detected level of generic *E. coli* was 2,742 MPN. Among the blueberry samples assayed (*n*=68), only 4.4% tested positive for generic *E. coli*, with the maximum level detected being 4 MPN generic *E. coli* per 100 grams of blueberries.

Significance: These results indicate a relatively high likelihood of detecting generic *E. coli* in surface irrigation water sources used on selected Michigan blueberry farms, and will be useful in establishing initial water quality profiles for these farms as required under the new FDA Produce Safety Regulation.

P1-20 Food Safety Risk Reduction by Use of In-line Disinfection for Contaminated Irrigation Water on Drip-irrigated Cabbage

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Introduction: Surface waters used for irrigation are prone to infrequent contamination and require close monitoring to avoid food safety hazards. The risk of using these sources for crop irrigation may be greatly reduced by in-line disinfection systems.

Purpose: Evaluate the ability of in-line water disinfection to reduce the transfer of *Salmonella* and Shiga-toxigenic *Escherichia coli* (STEC) to drip-irrigated cabbage.

Methods: A nearby pond containing populations of STEC and *Salmonella* was used as the surface water irrigation source for this study. Cabbage was grown in bare ground or plastic mulch covered plots that were drip irrigated with municipal water (negative control) or sand-filtered pond water (positive control). The sand-filtered pond water was also treated with ultraviolet light (47,000 μW s cm^{-2}; UV), 20 ppm chlorine dioxide (ClO_2), or 20 ppm peroxyacetic acid (PAA). Irrigation water samples were enumerated weekly for *Salmonella* and STEC. Harvested cabbage samples were enumerated on Chromagar STEC and XLT4, and selectively enriched. Positive results were validated using standard confirmation methods.

Results: Over 12 weeks, populations of *Salmonella* and STEC in untreated irrigation water fluctuated between not detected to 1.60 log CFU/100 ml and not detected to 2.88 log CFU/100 ml, respectively. UV, ClO_2, and PAA treatment methods were able to reduce *Salmonella* and STEC populations to below the detection limit (1 CFU/100 ml) on a regular basis. However, a single positive was observed with UV (4 CFU/100 ml *Salmonella* and 1 CFU/100 ml STEC) and ClO_2 (2 CFU/100 ml STEC). There were no *Salmonella*-positive cabbage samples in plots covered with plastic mulch and irrigated with UV or PAA treated water. Furthermore, there were no STEC-positive samples attributed to PAA/bare ground, chlorine dioxide/plastic mulch, or UV/plastic mulch plots.

Significance: Our findings suggest that in-line irrigation disinfection systems, when properly applied and monitored, can reduce the food safety risk associated with using surface water irrigation sources.

P1-21 Profiles of Postharvest Agricultural Water in Western Massachusetts

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Introduction: As produce has been linked with over 45 percent of all foodborne illnesses, it is important to develop methods to minimize the risk of these outbreaks. Postharvest agricultural water has been shown as a potential vector for cross-contamination. On farm use of sanitizer can reduce cross contamination, however, organic load strongly influences sanitizer effectiveness. Organic load, and its interaction with sanitizer is difficult to quantify, and only limited analysis of field conditions exist.

Purpose: Previous research determined that physiochemical properties of postharvest wash water affect sanitizer depletion. This research analyzed postharvest agricultural water from 12 locations in field sites within New England to profile the organic load generated during the production of small to medium farms. This provides a platform to help evaluate commercial produce sanitizers.

Methods: Samples were taken in triplicate from a variety of postharvest produce washing and cooling processes including hydrocoolers, tumble washers, and dunk tanks, processing with and without sanitizer. These samples were analyzed for physiochemical properties including turbidity and Chemical Oxygen Demand (COD), as well as coliform and *E. coli* loads using a Quanti-tray 2000.

Results: Different operations resulted in significant variance in organic loads. Coliform counts varied from 34.4 to over 2419 MPN. The turbidity ranged from 2.7 to 708 NTU. COD ranged from 8 to 112 mg/L. The COD, turbidity, and coliform levels did not interact in a linear manner. The highest measurements came from a tumble washer, and the lowest measurements came from a dunk tank in which water was changed often.

Significance: Organic load significantly affects sanitizer efficiency. It is imperative to understand the way postharvest processes generate organic load in order to apply sanitizer in a manner which minimizes chances of outbreaks.

P1-22 Investigating Indicators for Predicting the Presence of Foodborne Pathogens in the Irrigation Water of Produce Farms in the Lower Mainland of British Columbia, Canada

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Introduction: Foodborne outbreaks associated with fresh produce are increasingly recognized in North America. A potential etiological factor is contaminated irrigation water, which has been shown to transmit foodborne illness causing bacteria when applied to crops.

Purpose: Our objective is to improve current knowledge of factors and indicators associated with the occurrence of verotoxigenic *E. coli* (VTEC), *Salmonella*, and *Listeria monocytogenes* in irrigation water used in the Lower Mainland of British Columbia, Canada.

Methods: Water samples were collected bi-monthly between March and December 2015 from up to seven irrigation ditches in the Serpentine and Sumas watersheds of BC. VTEC colonies on water filters were detected using a verotoxin colony immunoblot (VT-IB) developed for the detection of all VTEC serotypes, and isolates were confirmed via multiplex PCR for virulence genes *stx1* and *stx2*. Detection of *Salmonella* and *L. monocytogenes* was completed using Health Canada Methods. Generic *E. coli* were enumerated using 3M Petrifilm Count Plates, and meteorological data were collected from Environment Canada records.

Results: VTEC, *Salmonella*, and *L. monocytogenes* were detected in 3 (2.7%), 3 (2.7%), and 14 (12.5%) of 112 samples, respectively. Pathogen occurrence was greater in the Serpentine watershed compared to the Sumas watershed (χ^2, *P* = 0.04), with *L. monocytogenes* also suggesting an increased occurrence in the Serpentine watershed (X^2, *P* = 0.07). Pathogen occurrence also correlated with lower temperatures (r_{pb} = -0.37), with positive samples being collected primarily during spring and fall. The number of generic *E. coli* correlated with the presence of both VTEC (r_{pb} = 0.538) and *L. monocytogenes* (r_{pb} = 0.442), but not with *Salmonella*. No correlations with precipitation were observed.

Significance: This research adds to the continued work to develop accurate risk models for these three pathogens, and may lead to economically feasible solutions for risk reduction at the farm level.

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P1-23 Persistence of Generic *E. coli* and Surrogate Pathogens on Strawberry Plants during Frost Protection Events: Challenges for the Implementation of the Newly Adopted FMSA Microbial Water Quality Standards

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Introduction: The microbial quality of water (MQW) used for frost protection is critical to reduce the risk of pathogen contamination of strawberries, especially during events close to fruit harvest where no further control measures exist to eliminate the risk. Meeting MQW standards and/or the alternative provisions adopted by the Produce Rule is essential to reduce pathogen contamination.

Purpose: To compare die-off rates of *E. coli* and surrogates of *Salmonella enterica, Listeria monocytogenes* and *E. coli* O157:H7 inoculated via sprinkler irrigation or spray inoculation to the surface of strawberries during frost protection events.

Methods: Strawberry plants were inoculated via sprinkler irrigation (*E. coli*) or by spray inoculation (Avirulent *Salmonella* Typhimurium, *E. coli* O157:H7 and *Listeria innocua*) during frost protection events with river, pond and well water. Average inoculum concentration was log 4.5 CFU/ml. Leaves/stems, berries and crown samples (150 – 300 g) were collected at 1, 3, 4, 6, 9 and 36-days post inoculation (DPI). Quantitative and qualitative recovery of each strain at harvest or during storage was achieved via selective/differential culturing methods and presumptive positives were confirmed via probe-based PCR.

Results: Populations of *E. coli* after 3-DPI using river, pond or surface water were below the limit of detection (LOD). *E. coli* persistence did not differ (P<0.05) between water sources. However, greater persistence was detected in the crown 36-DPI compared to leaves/stems and berries (100%, 36% and 7% respectively). After 12 days of storage at 8°C, 90% of fruit samples were positive for the inoculated *E. coli*. Die-off rate of *E. coli* was 0.12 log CFU/day after 36-DPI. After 4-DPI, all surrogate populations were at/below the LOD. After 9-DPI only the strawberry crowns were positive after enrichment for all inoculated strains.

Significance: Our findings suggest that current alternative provisions adopted by the Produce Rule to meet the necessary pathogen die-off rates after irrigation/frost protection events may need to be revised.

P1-24 Virulence Factors Detected by Whole Genome Sequence Analysis of Shiga Toxin-producing *Escherichia coli* Isolated from Irrigation Water

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Introduction: The characterization of microbiological hazards in irrigation water currently relies on prevalence data and broad assumptions about risks to human health. Information about the virulence of specific hazards can be derived from whole genome sequence (WGS) analysis.

Purpose: To compare virulence factors in Shiga toxin-producing *Escherichia coli* (STEC) isolated from irrigation waters in a defined geographic region of Canada with those found in human isolates.

Methods: Annotated genomes of STEC isolated from irrigation water in the Fraser Valley of British Columbia, Canada, were searched with VirulenceFinder v. 1.4 to detect Shiga toxin (*stx 1* and *stx 2)* and intimin gene (*eaeA*) allelic variants. Presence/absence of additional virulence factors were determined using the predictive genomics platform SuperPhy; statistical comparisons between water and human isolates were conducted using Fisher's exact test, and adjusted for multiple hypothesis testing using the false-discovery rate of Benjamini and Hochberg.

Results: A total of 47 STEC from 20 serotypes were recovered from water, including isolates from serogroups O157, O111, O26 and O103 bearing alleles for several *Stx* subtypes ($stx1_a$, $stx1_c$, $stx2_a$, $stx2_b$, $stx2_c$ or $stx2_d$) and allelic variants of the *eaeA* gene (β1, θ, ε, γ and ζ). Comparison with 312 human isolates with similar serotypes indicated that 17 of 380 known virulence factors were statistically over-represented among the water isolates, while 40 were statistically over-represented in the human isolates.

Significance: The STEC isolates included serotypes containing complements of virulence factors present in strains with known association to human disease. Hence, WGS analysis confirmed that STEC in irrigation waters in the region represent a potential threat to human health.

P1-25 Evaluation of the Microbial Quality of Agricultural Water Used in Pre-harvest Production on the Eastern Shore of Virginia

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Introduction: Several produce-borne outbreaks have been associated with use of contaminated water. The Food Safety Modernization Act-Produce Safety Rule regulation provides standards for the microbial quality of agricultural water.

Purpose: This study evaluated the microbial quality of surface agricultural water used in pre-harvest production on the Eastern Shore of Virginia.

Methods: Water samples (1 L) from 20 agricultural ponds were collected during the 2015 growing season (n=200). Total aerobic bacteria, total coliforms, and generic *Escherichia coli* were enumerated for each sample. Population levels of each microorganism were calculated in log CFU/100 ml. Samples were also enriched for *Salmonella*. Environmental and meteorological factors were analyzed for their association with the detection of a *Salmonella*-positive water sample.

Results: Currently, 17 of the 20 ponds met the FSMA-Produce Safety Rule standards for surface agricultural water. Three ponds did not meet the standards because the statistical threshold value exceeded 410 CFU/100 ml of generic *E. coli*. *Salmonella* was detected in 19% of water samples (38/200). Interestingly, 17 of the 20 ponds were *Salmonella*-positive at least once during the growing season. Total aerobic bacteria, total coliforms, and generic *E. coli* levels were not associated with the detection of *Salmonella* in water samples. Furthermore, farm was significantly associated with the likelihood of detection of *Salmonella*.

Significance: Minimal data exists on surface water metrics in Virginia. Our findings provide crucial data that will assist growers with science-based evaluation of their water and implementation of strategies that reduce the likelihood of produce contamination.

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P1-26 Effectiveness of Ultraviolet (UV-C) Light Treatment on Reducing Microbial Levels from Surface Water Used for Irrigation of Cantaloupes

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Introduction: Contaminated surface water is a potential source of microbial contamination. Producers are looking for irrigation water treatments that do not leave any chemical residue in the water.

Purpose: This work evaluated the efficacy of ultraviolet (UV)-C light on pathogen risk reduction from surface water used for irrigation of cantaloupes in an agricultural setting.

Methods: Replicated cantaloupe plots (12 plots, 3/treatment, plot size 5' by 15', 10 plants/plot) were irrigated (spray or drip irrigation) with UV-C light treated or non-treated water. A mixture of pond and well water (1000 L) (UV transmittance rate 53.74±2.12) collected in a tank was inoculated with a cocktail of generic *E. coli* (ATCC 23716, 25922 and 11775). The inoculated water (7.4 log MPN/100ml) was then treated with UV-C light (16 to 120 mJ/cm^2) by passing through a UV-C treatment unit PMD50C1/4.

Results: Significant reduction (P<0.05) of generic E. coli (>4.5 log MPN/100 ml was achieved with lower doses (10 to 20 mJ/cm^2) and below the detectable limit of the test for doses above 60 mJ/cm^2. The generic *E. coli* counts on cantaloupe irrigated with UV-C treated or non-treated water were similar (2.57±0.12 and 2.67±0.11 log MPN/cantaloupe). Cantaloupes harvested from drip-irrigated plots were significantly lower in counts (2.72±0.12 log MPN/cantaloupe) as compare to sprinkle irrigated plots (3.97±0.15 log MPN/cantaloupe). Within the drip irrigated treatment plots, cantaloupe harvested next to the drip emitter was higher in counts by at least 1.2 log as compared to cantaloupes far from the drip. In the first trial, a significant die-off of generic *E. coli* was observed on cantaloupe surfaces within three days from the last day of irrigation; however, levels were similar for five days in the second trial.

Significance: Our results indicated UV-C light as an effective antimicrobial treatment for microbial risk reduction of surface irrigation water, but influence on generic *E. coli* levels on cantaloupe surface was unclear.

P1-27 Spread of *Escherichia coli* O157:H7 during Flume Washing and Drying of Fresh-cut Romaine Lettuce

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❖ ***Developing Scientist Competitor***

Introduction: The microbiological safety of leafy greens remains a concern as evidenced from recent outbreaks.

Purpose: Consequently, this study assessed the spread of *Escherichia coli* O157:H7 during pilot-scale washing and drying of fresh-cut Romaine lettuce.

Methods: Using inoculated radicchio as a colored surrogate for uninoculated Romaine lettuce, radicchio leaves (~5 x 5 cm) were spot-inoculated at ~10^{-1}, 10^1 and 10^3 CFU/leaf and mixed with uninoculated Romaine lettuce leaves (~5 x 5 cm) to obtain 5 kg batches having inoculated:uninoculated product ratios of 0.5:100, 1:100, 5:100 and 10:100. After 2 minutes of flume washing in sanitizer-free water followed by shaker table dewatering and centrifugal drying in a pilot-scale processing line, all radicchio leaves were removed. Each batch was then divided into ~20 225-g samples which were analyzed for presence/absence of *E. coli* O157:H7 using the *E. coli* O157 GeneQuence method (Neogen Corp, Lansing, MI). The percentage of *E. coli* O157:H7 lost from radicchio during processing was determined by direct plating before and after processing with *E. coli* O157:H7 populations also assessed in 50 ml water samples by membrane filtration.

Results: Based on triplicate experiments, lower radicchio inoculation levels led to decreased *E. coli* O157:H7 transfer to Romaine lettuce during processing (P<0.05). All lettuce samples yielded *E. coli* O157:H7 when radicchio was inoculated at 10^3 CFU/leaf. When radicchio was inoculated at 10^1 CFU/leaf, the percentage of positive samples decreased from 100-90.5% to 100-81.0%, 90.5-76.2% and 85.7-28.6% for inoculated:uninoculated ratios of 10:100, 5:100, 1:100 and 0.5:100 while at 10^{-1} CFU/leaf, 38.1-4.8%, 9.5-0%, 4.8% and 19-0% of the samples were similarly positive for the same inoculated:uninoculated ratios. Within each inoculation level, no significant differences (P>0.05) were found among the four inoculated:uninoculated product ratios. *E. coli* O157:H7 was non-quantifiable in the water samples.

Significance: These findings will provide important data for improving exposure assessment in risk assessments for leafy greens.

P1-28 Modified Coring Tool Designs Reduce Iceberg Lettuce Cross-contamination

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Introduction: Contaminated coring tools can cross-contaminate iceberg lettuce heads during coring.

Purpose: Modifications in coring tool designs were evaluated for reduced cross-contamination of iceberg lettuce heads.

Methods: The original tool had a straight bottom edge core knife attached with welding. Modifications were done to the welding, coring knife and length. Design D2L2 (Design, Length) was similar to original design but did not have a welded edge. Design D3L1 had an angled edge bottom while D1L2 had a short front straight bottom edge. The blade length of original tool and modified designs- D1L2, D2L2, D3L1 were 7.5, 10.5, 10.5 and 7.5 cm, respectively. All tools were surface contaminated with *Escherichia coli* K12 and then used to core 100 lettuce heads consecutively. The cored regions were scraped from all 100 heads and tested for *E. coli* after enrichment. All tool designs were dipped in bioluminescent *Salmonella* Newport contaminated- water, water with lettuce extract, and tryptic soy broth (TSB) for 2 min and imaged using EMCCD camera to observe bacterial attachment.

Results: Absence of welding from original tool design resulted in the highest reduction in *E. coli* transfer. Original tool and welding free D2L2 resulted in 91±9% and 44±11.9% *E. coli* positive lettuce heads, respectively. Elimination of the welded region resulted in a progressive decrease of *E. coli* on cored lettuces. Lettuces 80-100 cored with original tool and D2L2 were 86.7% and 26.7% positive for *E. coli*, respectively (P<0.05). Incorporation of a short front straight edge resulted in 65.6±5.6% (D1L2) of cored lettuce heads being positive for *E. coli*. Biophotonic imaging indicated that immersion in water containing lettuce extract and TSB caused increased attachment of *Salmonella* to tool surface compared to water.

Significance: Modified coring tool designs can help reduce cross-contamination of cored iceberg lettuce heads. Biophotonic imaging can be used to visualize bacteria attached on produce surfaces.

Poster

P1-29 Susceptibility of Environmental *Salmonella* Strains to Medium and Long Chain Fatty Acids Found Naturally in Tomato Fruit

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Introduction: Tomato fruit produce a wide variety of saturated and unsaturated fatty acids, which vary by cultivar and maturity. A number of fatty acids can have antimicrobial activity. Serotypes of *Salmonella enterica* show differential growth on tomato fruit, possibly in response to tomato fruit and fruit exudate chemical composition.

Purpose: The susceptibility of tomato outbreak-associated *Salmonella* serotypes to medium and long chain fatty acids known to be present in tomato fruit and exudates was assessed to determine whether fatty acids play a role in *S. enterica* fruit surface colonization.

Methods: Minimum inhibitory concentrations (MIC) of the fatty acids pelargonic (C9:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), margaric (C17:0), stearic (C18:0) and oleic (C18:1) acids against *Salmonella* serotypes Newport, Javiana and Typhimurium were determined. The environmental test microorganisms were isolated from mid-Atlantic tomato farms. Stock solutions were suspended in water, water containing 0.001% *Quillaja* saponin or dimethyl sulfoxide (DMSO). The resazurin microtiter assay was used to determine MIC. Concentrations of 1M, 500mM, 250mM, 125mM and 62.5mM were suspended in Isosensitest Broth containing resazurin in 96-well plates and inoculated with 6 log CFU/ml *Salmonella*. The plates were incubated for 16-18 h at 37°C. Color change indicated bacterial growth. The lowest concentration of fatty acid where no color changed was observed was considered the MIC.

Results: Differences in MIC were observed based on fatty acid, serotype and suspension medium. All serotypes demonstrated highest sensitivity to pelargonic acid. In water, water with saponin and DMSO, *Salmonella* Newport showed MICs of 125mM, 187.5(±88.4) mM, and 62.5mM, respectively, *Salmonella* Javiana demonstrated MICs of 250mM, 62.5mM and 31.25mM, respectively and *Salmonella* Typhimurium exhibited MICs of 250mM, 62.5mM, and 46.875(±22.1) mM, respectively. In presence of saponin, lauric acid (1M) also inhibited *Salmonella*.

Significance: Fatty acids in tomato fruit could result in inhibition of *Salmonella* growth in a serotype-dependent manner.

P1-30 Novel Photosensitizer Application on Tomatoes and Leafy Greens Results in Hydrogen Peroxide Formation

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Introduction: Photosensitizers generate reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) when exposed to UV-light, resulting in antibacterial activity against *Escherichia coli* in solution. These compounds show promise as pre- or post-harvest treatments to reduce enteric and plant pathogens.

Purpose: This study aimed to determine whether H_2O_2 is generated on the surface of produce following application of solutions of the generally recognized as safe (GRAS) compounds gallic and benzoic acids, and UV-exposure.

Methods: Harvested tomato fruit and romaine lettuce leaves in pots were immersed in 10 mM solutions of gallic acid, benzoic acid, or water (control). The photosensitizer-treated tomatoes and lettuce were exposed to visible light in a plant growth chamber for 16 h light at 25°C and 8 h darkness at 18°C. Produce was analyzed for H_2O_2 production after 4 h and 24 h using the FOX (ferrous ion oxidation in presence of xylenol orange) assay. Change in absorbance due to H_2O_2 presence was measured at 560 nm using a spectrophotometer. Color change of produce was also evaluated after the treatments.

Results: H_2O_2 production measured after 4 h on tomato fruit that was treated with benzoic acid resulted in an OD_{560} of 0.69±0.11, and was significantly higher than the water control (P<0.05). Exposure of tomato to gallic acid resulted in an OD_{560} of 0.34±0.09 after 4 h, which was similar to the control. After 24 h, levels had returned to baseline. Lettuce treated with benzoic acid resulted in OD_{560} of 0.27±0.02 and 0.26±0.03 after 4 and 24 h, respectively, exhibiting no increase in H_2O_2 generation relative to the control. No impact on color change or visual quality in tomato occurred after treatments. Deterioration in lettuce quality was observed following benzoic acid, but not gallic acid treatment.

Significance: Benzoic acid application to tomatoes followed by UV exposure generated the antimicrobial compound H_2O_2 without compromising tomato quality.

P1-31 Study Using Indicator Microorganisms in Evaluating the Efficiency of Peroxyacetic Acid Wash in Leafy Greens Processes

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Introduction: Determining microbial reduction of indicator microorganisms in wash water samples is one of the measures to validate efficacy of sanitizer washing of fresh produce. Peroxyacetic acid (PAA) is becoming more popular for leafy greens washes; however, the feasibility of using indicators to evaluate PAA washing is unclear.

Purpose: To determine the difference between indicator microorganism groups and *E.coli* O157:H7 in PAA inactivation in water, and level of cross-contamination in lettuce wash using PAA-water.

Methods: Inactivation of indicator microorganisms [total viable count (TVC), *Enterobacteriaceae*, total coliforms] and *E. coli* O157:H7 was determined when exposed to PAA (0-150 ppm) in wash water with lettuce juice added as additional organic load. Cross-contamination of indicator microorganisms and *E. coli* O157:H7 from inoculated lettuce (~ 5 log CFU/cm^2) to water was evaluated in 50-ml scale washing with different level of PAA (20, 40, 60, and 80 ppm). Bacterial survival or transfer to wash water was quantified by plating on appropriate media.

Results: For microbial inactivation using PAA, fewer numbers of *Enterobacteriaceae* and total coliforms than *E. coli* O157:H7 survived when exposed to the same levels of PAA. These two indicator groups were completely inactivated at 40 ppm, while *E. coli* O157:H7 had a reduction of only 0.7 log CFU/ml. At 80 ppm PAA, *E. coli* O157:H7 was completely inactivated; however, TVC was inactivated only when PAA increased to 150 ppm. For lettuce wash with 60 ppm PAA-water, approximately 1 log CFU/ml *E. coli* O157:H7 was detected in wash water after 60 second wash while *Enterobacteriaceae* and total coliforms were not detectable. After 80 ppm PAA wash, *E. coli* O157:H7 was completely eliminated and transfer of TVC to wash water was still observed.

Significance: TVC, *Enterobacteriaceae* and total coliforms may not be good indicator groups for evaluating control of *E. coli* O157:H7 during lettuce washing using PAA.

Poster

P1-32 Evaluating the Efficacy of Ozone and Modified Atmosphere Packaging at Extending the Lag Phase of Native Microflora on Vegetables Stored at Non-optimum Temperatures

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Introduction: Cold storage is not always readily accessible for small vegetable growers, as access to refrigerated trucks and cooling facilities is often limited. This can be particularly problematic for farmers selling at wholesale markets, as quality and safety of the product may be negatively affected when stored at non-optimum temperatures.

Purpose: This research was conducted to determine if a postharvest ozone wash and passive modified atmosphere packaging (MAP), either alone or in combination, can restrict the growth of microbial populations on vegetables stored at non-optimum temperatures.

Methods: Broccoli, spinach and asparagus were collected at harvest from local vegetable farmers in Kansas. Products were either 1) unwashed, 2) washed in 4°C water (1 minute) or 3) washed in ozonated water (1.0-1.5 ppm for 1 minute) prior to storage in an open produce bag or a MAP bag at 13°C with 85% RH. As an indication of product safety, populations of yeasts, molds, psychrotrophs, total aerobic microorganisms and coliforms were quantified on days 0, 3, 5 for asparagus and broccoli and days 0, 3, 6, 9, 12, 15, 18 for spinach.

Results: An atmosphere of 7% CO_2 and 6% O_2, 4% CO_2 and 7% O_2 and 12% CO_2 and 3% O_2 was equilibrated in MAP bags containing spinach, asparagus and broccoli, respectively. All treatments were statistically the same (P > 0.05) for all microorganisms and products evaluated.

Significance: A post-harvest ozone wash and MAP packaging, implemented as either separate interventions or in combination, was not effective at extending the lag phase of native microflora on spinach, asparagus and broccoli stored at non-optimum temperatures. Future research is necessary to determine if 1) increasing the concentration of ozone and/or the contact time, with or without MAP packaging, would be more efficacious and 2) other postharvest washing interventions are more efficacious.

P1-33 In-package Inhibition of *E. coli* O157:H7 on Bulk Romaine Lettuce Using Cold Plasma

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Introduction: Dielectric barrier discharge atmospheric cold plasma (ACP) is an innovative method for in-package decontamination of fresh produce. However, data is lacking for ACP decontamination of bulk leafy produce in commercial packages.

Purpose: This study evaluated ACP treatment uniformity and efficacy when applied to bulk Romaine lettuce leaves, inoculated with *E. coli* O157:H7and stacked in a commercial plastic clamshell container. Color, surface morphology, carbon dioxide (CO_2) generation, and weight loss of the leaves were also determined for leaves stacked in different positions in the container.

Methods: Romaine lettuce samples, washed and inoculated with *E. coli* O157:H7 (~6 log CFU/g lettuce), were packed in a model bulk packaging configuration: three rows and 3 or 5 layers (container: 14.8 × 12.8 × 2.7 cm). Packaged lettuce was treated with ACP (gap distance: 3.0 cm) at 61.2 kV (1.3 A) for 10 min. Leaves were removed in order and analyzed for *E. coli*O157:H7 inhibition, color (Hunter Lab), and weight loss of the samples (three replications). **Results** were correlated with the leaf position within the bulk stack, i.e., proximal to or distal from the electrodes in this prototype.

Results: ACP reduced *E. coli* O157:H7 on packaged lettuce by 0.4±0.2 - 0.8±0.2 log CFU/g lettuce, with no significant correlation to where the leaves were located in each stacked configuration (P > 0.05). Respiration, leaf weight, and leaf color (L^*, a^*, and b^* values) were not significantly different (P > 0.05) for leaves from any position in the bulk stack, regardless of the number of layers.

Significance: ACP is a promising method for inactivation of *E. coli* O157:H7 on Romaine lettuce leaves within commercial packages as a terminal processing step. Container headspace volume and orientation of lettuce with respect to discharge electrodes are key areas for further research and development for this technology.

P1-34 Suitability of *Enterobacter aerogenes* and Avirulent *E. coli* as Surrogates for Pathogenic *E. coli* during Washing of Cut Lettuce

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❖ ***Developing Scientist Competitor***

Introduction: Cross-contamination during washing has been identified as an important variable in controlling risk for leafy greens. Research with pathogens at pilot plant scale or larger is problematic, so non-pathogen or avirulent surrogates are needed.

Purpose: This study quantifies cross contamination by *Enterobacter aerogenes*, avirulent *E. coli* O157:H7, pathogenic *E. coli* O157:H7 during lettuce washing.

Methods: The experimental variables were post-inoculation drying time (10 min vs. 2 h), water volume (100 ml vs. 1L), and ratio of inoculated to non-inoculated lettuce pieces (1:5 vs. 1:20). Overnight cultures were centrifuged and resuspended in peptone water prior to inoculation. A single piece of baby romaine lettuce was spot inoculated with ~ 6 log CFU of bacteria, dried, and washed with the non-inoculated pieces in a stainless-steel bowl for 30 seconds. Bacterial reduction and cross-contamination were quantified. Data were analyzed using ANOVA in Microsoft Excel.

Results: In every case, most (~99%) of the bacterial contamination was transferred to the water, and ~1% of the inoculated bacteria transferred to the non-inoculated pieces. Water volume (P=0.02) and drying time (P<0.0001) had a significant effect of log-reduction. The ratio of inoculated to non-inoculated leaves and organism type did not have a significant effect of log reduction (P>0.05). Water volume (P<0.001), drying time (P=0.04) had a significant effect on log % transfer. Ratio of inoculated to non-inoculated lettuce did not have a significant influence on log % transfer (P=0.12). *E. aerogenes* showed significantly different transfer than pathogenic *E. coli* O157:H7 (P<0.001) but avirulent *E. coli* O157:H7 transfer results were not significantly different than pathogenic *E. coli* O157:H7 (P=0.1).

Significance: These results show that avirulent *E. coli* O157:H7 can be used as surrogate for pathogenic *E. coli* O157and that water volume and drying time are important variables to consider for leafy green cross-contamination research.

P1-35 Minimum Effective Concentrations of a New Fresh Produce Wash (*First Step*+ *10*), Compared to Chlorine at Inactivating Foodborne Pathogens in Rinse Water

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Introduction: We previously reported inactivating *E. coli* O157:H7 (Ec), *Salmonella* and *Listeria monocytogenes* (Lm), by a new produce wash, *First Step+ 10* (FS+10) at concentrations of 0.5, 0.6, 0.8, 1.0, 1.2, and 1.6%. Chlorine concentrations in rinse water frequently fall to low levels (e.g., 10-16 ppm), due to inactivation by organics.

Purpose: Determine the minimal FS+10 concentrations effective at inactivating foodborne pathogens in rinse water, compared to low levels of chlorine.

Methods: An organic interference substance (bovine albumen) was added to multi-strain suspensions of Ec, *Salmonella,* and Lm, at 0.03% (BSEN 1276 method). Two additional interference substances (5% fresh-squeezed apple juice (AJ) or 2.5% fresh lettuce juice (LJ)) were also tested. The three pathogens were challenged with 0.2, 0.3, and 0.4% of FS+10 (for bovine serum, and 0.4 and 0.5% for juices), as well as with 10 and 16 ppm chlorine (citric acid-adjusted to pH 7.0).

Results: All concentrations of FS+10 (0.2, 0.3, and 0.4%) inactivated >5 logs of all pathogens, with no statistical difference between treatments (P<0.05, Scheffe). Average log reductions for the three concentrations of FS+10 were Ec (all >5.93), *Salmonella* (5.14, >5.61, >5.69), and Lm (all >5.7). FS+10 inactivated statistically greater numbers of all pathogens than 16 ppm chlorine, which was > than that inactivated by 10 ppm chlorine. Average log reductions for 16 and 10 ppm chlorine were Ec (1.79, 0.64), *Salmonella* (2.72, 1.20), and Lm (2.77, 1.22). In the presence of 5% AJ, reductions for 0.5 and 0.4% concentrations of FS+10, respectively, were Ec (6.25, 5.06), *Salmonella* (>6.78, 6.18), and Lm (>6.69, 2.94). Log reductions in the presence of 2.5% LJ, were Ec (>6.78, 4.99), *Salmonella* (>6.87, >6.56), and Lm (6.12, 2.49).

Significance: FS+10 is capable of inactivating pathogens in wash waters with the interference substances tested here, which may assist producers in their food safety hygiene plans.

P1-36 Roles of Extracellular Polysaccharides of *Escherichia coli* O157:H7 in Survival of the Enteric Pathogen on *Arabidopsis* and Lettuce

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Introduction: Bacterial polysaccharides may play important roles in interactions of human enteric pathogens with plants; facilitating attachment and colonization on plant surfaces.

Purpose: The objective of this study was to investigate the influence of *E. coli* O157:H7 extracellular polysaccharides (cellulose, colanic acid, and lipopolysaccharide) on survival/colonization on plants.

Methods: *Arabidopsis thaliana* ecotype Columbia (Col-0) and Romaine lettuce were used in bacterial colonization experiments to compare survival trends on a plant model and produce commodity, respectively. Four week-old *Arabidopsis* plants and lettuce plants were dip-inoculated with wild-type *E. coli* O157:H7 strain 86-24 and its corresponding mutants (cellulose-deficient; *ΔyhjN*, colanic acid-deficient; *ΔwcaD*, and truncated LPS; *Δwaal*). Initial populations on day 0 were approximately 10^6 CFU/g leaf tissue. At days 1, 3, and 5 post-inoculation, three plants for each treatment were harvested and *E. coli* O157:H7 populations determined by plating plant homogenates onto Tryptic Soy Agar supplemented with appropriate antibiotics. Extracellular polysaccharide production of each strain was quantified using the hot phenol-water extraction method.

Results: On day 5 post-inoculation, the populations of colanic acid-deficient (2.79 log CFU/g) and LPS truncated (2.59 log CFU/g) mutants on *Arabidopsis* were significantly (P < 0.05) lower than the population of the wild-type strain (3.84 log CFU/g). Wild-type *E. coli* O157:H7 survived better on both *Arabidopsis* and lettuce than colanic acid-deficient and LPS truncated mutants at day 1 and 5 post-inoculation. The wild-type strain produced significantly greater (P< 0.05) amounts of extracellular polysaccharide than the colanic acid and LPS mutants.

Significance: This study demonstrates that *E. coli* O157:H7 polysaccharides colanic acid and LPS may impact survival of the pathogen on plants depending on the amount of extracellular polysaccharide produced.

P1-37 The Effect of pH and Temperature on Chlorine Inactivation of *Escherichia coli* O157:H7

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Introduction: Wash-water chlorination is a critical control step to prevent microbial cross-contamination during post-harvest processing of fresh produce. Post-harvest washing may operate at varying temperatures and pH levels for different produce. Therefore, inactivation kinetics of chlorine at different pH and temperatures needs to be determined to optimize washing operations.

Purpose: Evaluate the chlorine inactivation of *E. coli* O157:H7 as affected by pH and temperature variation.

Methods: Phosphate buffer was prepared and adjusted to two pH levels (6.5 and 8.0). Inactivation trials were performed at 0, 0.25, 0.5, and 1 ppm free chlorine, at ambient temperature and 4°C. Chlorinated samples (30 ml) were inoculated with 6 log CFU/ml of *E. coli* O157:H7 and mixed for 3, 10, 20, or 30 seconds, then neutralized using sodium thiosulfate. Levels of *E. coli* O157:H7 in the samples were determined by plate counts.

Results: Reduction in *E. coli* O157:H7 counts increased with increasing free chlorine levels, decreasing pH, and increasing temperature. At 4°C and 1 ppm free chlorine, after 30 second exposure, the extent of pathogen reduction increased from 0.45 ± 0.19 at pH 8.0 to 7.36 ± 0.34 log CFU/ml at pH 6.5. At pH 6.5, the difference in log reduction between 4°C and ambient temperature was approximately 2 log after 30-second exposure to 0.25 ppm of chlorine. At pH 8.0, the greatest difference occurred at 1 ppm chlorine, where treatment for 30 seconds at 4°C showed a log reduction of 0.45 ± 0.19 log CFU/ml while treatment at the ambient temperature resulted in a log reduction of 5.70 ± 0.38 log CFU/ml.

Significance: Both pH and temperature affect chlorine inactivation of *E. coli* O157:H7. Small pH variation at near-neutral pH range significantly impacts chlorine inactivation kinetics and should be tightly controlled for optimal wash water management.

P1-38 Gaseous Ozone and Bacteriophage Act Synergistically against *Escherichia coli* O157:H7 on Spinach Leaves

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❖ ***Developing Scientist Competitor***

Introduction: Contamination of fresh produce with enterohemorrhagic *Escherichia coli* (EHEC) remains one of the leading causes of foodborne disease outbreaks in the United States. Low infective dose of EHEC and minimal post-harvest processing of fresh produce necessitate the search for effective disinfection approaches to protect the public against disease outbreaks and the produce industry from economic losses.

Purpose: In this study, we investigated the efficacy of gaseous ozone during vacuum cooling and subsequent application of bacteriophage for inactivation of *E. coli* O157:H7 on fresh produce.

Methods: Spinach leaves were spot-inoculated with low or high concentrations of *E. coli* O157:H7 (5.3 or 6.6 log CFU/g, respectively). Inoculated leaves were subjected to gaseous ozone during vacuum cooling as follows: initially 28.5 in. Hg vacuum was applied, followed by gaseous ozone treatment at 1.5 g ozone/kg ozone-oxygen gas mix, at 10 psig vessel pressure, and 30 min holding time. Ozone-treated leaves were sprayed with *E. coli* phage at 8.3 log PFU/g, then held for five minutes.

Results: At 5.3 log CFU/g inoculum level, gaseous ozone alone significantly (P < 0.05) reduced *E. coli* population by 3.1 log CFU/g when compared to inoculated untreated control. Sequential treatment of ozone and bacteriophage significantly (P < 0.05) reduced *E. coli* populations below the detection limit of the enumeration method (i.e., < 1 log CFU/g). At 6.6 log CFU/g, gaseous ozone and sequential treatments significantly (P < 0.05) reduced *E. coli* counts by 1.8 and 5.2 log CFU/g, respectively.

Significance: Regardless of inoculum size, sequential treatments considerably decreased the risk of contamination of fresh produce with *E. coli* O157:H7. Considering the realistic low contamination levels of fresh produce and low infective dose of *E. coli*, sequential application of gaseous ozone and bacteriophage seems a promising post-harvest disinfection method to secure the safety of produce.

P1-39 Quality Analysis of Produce Wash Water in Commercial Flume Wash System

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Introduction: Reuse of wash water during post-harvest processing of fresh-cut produce, requires critical monitoring and maintenance of water quality to ensure food safety of the washed products, as free chlorine concentration in flume system wash water is rapidly depleted by high loads of organic matter introduced with the produce.

Purpose: The objective of this study was to evaluate water quality variation during routine operations of fresh-cut produce processing, and analyze multiple factors influencing the effectiveness of antimicrobial washes, with a view toward developing practical guidelines for validation and monitoring.

Methods: Research was conducted in collaboration with commercial processing facilities. We collected in-line water samples during regular operation for three types of leafy-greens (iceberg lettuce, romaine lettuce, and cabbage), and analyzed the water quality on-site, including pH, free chlorine, total chlorine, chemical oxygen demand (COD), turbidity, and total dissolved solids (TDS).

Results: Water quality declined continuously during washing operations. The greatest decrease in water quality occurred during diced cabbage wash, with increased COD (max.14870 mg/L), turbidity (max. 281 NTU) and TDS (max. 4750 mg/L), along with fluctuations in free chlorine, total chlorine, and relatively stable pH. COD values correlated strongly with turbidity and TDS. The best correlation was present in romaine lettuce wash water (Pearson's r=0.95 for COD vs. turbidity) (Pearson's r=0.97 for COD vs. TDS). Although free chlorine levels were independent of COD, turbidity and TDS, total chlorine levels correlated well with COD, turbidity and TDS in romaine lettuce wash water (Pearson's r=0.69, 0.61, 0.73, respectively). Further analysis showed that COD increases linearly with turbidity (R^2 =0.9075 for romaine lettuce) and TDS (R^2=0.9408 for romaine lettuce).

Significance: The results are useful for regulatory agencies and the produce industry in developing guidelines to maintain the quality and food safety of leafy-greens processed by chlorine-based wash systems.

P1-40 A Preliminary Investigation into the Efficacy of Potassium Bisulfate as a Pre-harvest Intervention to Control the Foodborne Pathogen Surrogates *Listeria innocua* and *Escherichia coli* on Lettuce

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Introduction: Pathogenic *Escherichia coli* and *Listeria monocytogenes* have been increasingly implicated in produce-related foodborne outbreaks. Many outbreaks originate on the farm; thus, research evaluating the effectiveness of pre-harvest antimicrobial interventions is necessary.

Purpose: The goal of this study was to determine the efficacy of potassium bisulfate in reducing foodborne pathogen surrogates on lettuce in the pre-harvest environment.

Methods: Pelleted lettuce (*Lactuca sativa*) was grown in a greenhouse until maturation and then inoculated with overnight cultures of either *Escherichia coli* or *Listeria innocua* by hand spraying onto the plant surface until the drip point. Inoculated lettuce was randomly allocated to the control (untreated lettuce), water control (plants sprayed with water until the drip point), or treatment (0.25% potassium bisulfate sprayed until the drip point) groups prior to treatment application at either two days or seven days prior to harvest. Immediately following harvest, samples were analyzed for *Escherichia coli* or *Listeria innocua* by plating onto MacConkey and Modified Oxford agar, respectively.

Results: When applied one week before harvest, potassium bisulfate significantly reduced *Escherichia coli* populations on inoculated lettuce by 1.3 log CFU/g (P=0.0215), 1.2 log CFU/g (P=0.0422) and 1.3 log CFU/g (P=0.0226) in comparison to the untreated control, 7-day water control and 2-day water control, respectively. No statistically significant differences were detected for all treatments applied to *Listeria innocua* inoculated lettuce.

Significance: This research suggests that potassium bisulfate may be a suitable pre-harvest intervention to reduce pathogenic *Escherichia coli* on lettuce. The modest reduction in *Escherichia coli* populations suggests that efforts to optimize the intervention (e.g., application with a surfactant to improve leaf surface coverage) may be warranted.

P1-41 Sanitizer Tolerance and Surface Attachment Differences among Persistent and Non-persistent *Listeria monocytogenes* Strains Isolated from a Mushroom Slicing and Packaging Environment

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Introduction: A recent 13-month longitudinal survey of a mushroom processing environment revealed 4 virulence types (VTs) of *Listeria monocytogenes* using multi-virulence-locus sequence typing; predominant persistent clone VT11, non-predominant persistent clone VT105, and transient clones VT107 and VT56.

Purpose: Isolation of VT11 after cleaning and sanitizing indicated the need to evaluate the efficacy of the quaternary ammonium chloride (QAC) sanitizer used in the facility as well as the ability of VT11 to attach to surfaces.

Methods: Strain differences for QAC tolerance were therefore compared by determining minimum inhibitory concentrations (MIC) and decimal reduction times (D_{200ppm}). Mean log density (MLD) values at 48 h for each strain were compared for surface attachment on stainless steel (SS) and concrete surfaces.

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Results: MIC values for all VTs were 4.5 ppm and D_{200ppm} ranged from 0.76 to 2.10 s. No significant differences in D_{200ppm}were found for 20 h cells; although, the mean value for 7 d long term survival (LTS) VT11 cells was significantly higher than other VTs ($P \leq 0.05$). Overall, MLD values were significantly higher ($P \leq 0.05$) on concrete (7.10 ± 0.49) compared to SS (5.21 ± 0.81) surfaces. MLD values on concrete were significantly lower ($P \leq 0.05$) for VT105 (6.41 ± 0.25) compared to VT11 (7.27 ± 0.33), VT107 (7.22 ± 0.26) and VT56 (7.32 ± 0.33). SS adherence was significantly higher ($P \leq 0.05$) for VT11 (5.73 ± 0.30) and VT107 (5.87 ± 0.68) than VT105 (4.47 ± 0.69) and VT56 (4.76 ± 0.44). These results suggest that the observed predominance and persistence of VT11 over long periods of time may at least be partially explained by increased tolerance of the LTS phase of this strain to QAC.

Significance: These results are useful for providing guidance on targeted interventions to reduce *L. monocytogenes* contamination risks in mushroom and other fresh produce facilities.

P1-42 Evaluating Survival of *Salmonella* Newport on Iceberg Lettuce Coring Tools and the Efficacy of Plant Antimicrobials and Organic Sanitizers

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Introduction: Foodborne outbreaks associated with leafy greens are attributed to many factors including cross-contamination between harvesting equipment and leafy greens.

Purpose: The objectives of this study were to determine specific locations on iceberg lettuce coring tools prone to attachment by *Salmonella* and evaluate efficacy of sanitizers on these tools.

Methods: Three different designs (original and modified) of coring tools were inoculated with *Salmonella* Newport overnight culture (8 log CFU/ml) and treated with deionized water (DI water), 50 ppm bleach, 3% hydrogen peroxide, 5% Chico wash, 0.1% Oregano oil, or 0.4% SaniDate 5.0 for 5 min. Four locations on tools were sampled before and after treatment for enumeration of surviving *Salmonella.*

Results: *Salmonella* populations of 6.35±0.26, 6.31±0.10 and 6.26±0.25 log CFU/cm^2 attached onto the original, modified design 1, and modified design 2 coring tools, respectively. Locations 1 and 4 on the original tool had the highest populations of *Salmonella*, 6.47±0.18 and 6.64±0.20 log CFU/cm^2, respectively. When comparing the efficacy of sanitizers, SaniDate® had the highest *Salmonella* reductions of 5.86±0.58-6.23±0.28 log CFU/cm^2. Treatments with 3% H_2O_2 and 0.1% oregano oil were comparable which yielded reductions of 5.82±0.38 and 5.68±0.39 log CFU/cm^2, respectively. The average reductions due to 50 ppm bleach were 2.72±0.69-3.65±1.65 log CFU/cm^2. The wash water after treatment with each sanitizer was sampled after enrichment to check for *Salmonella* survivors; however, no survivors were detected (except DI water) suggesting that these organic sanitizers and plant antimicrobial have bactericidal effects on *Salmonella*.

Significance: The results indicate that organic sanitizer SaniDate and plant antimicrobial oregano oil, showed better efficacy against *Salmonella* on coring tools compared to 50 ppm bleach. The results could provide guidelines regarding the areas on coring tools that may require rigorous sanitization and also suggest potential alternative organic sanitizers that could be more effective than those currently used against *Salmonella* for application on coring tools.

P1-43 Effect of Novel Sanitizers on Murine Norovirus on Romaine Lettuce Combined with High Power Ultrasound

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❖ ***Developing Scientist Competitor***

Introduction: Human norovirus (HuNoV) is a leading cause of foodborne outbreaks in fresh-cut produce. Sanitizers have been used in food industry to prevent cross-contamination; however, most of them have proven to be less effective on HuNoV compared to bacterial agents. High-Power-Ultrasound (HPU) has a potential to improve the efficacy of sanitizers and demonstrated to provide additional removal of bacterial pathogens from fresh-cut produce surface.

Purpose: This study aimed to determine if novel sanitizers in combination with HPU could effectively remove murine norovirus (MNV-1), a HuNoV surrogate from the surface of romaine lettuce.

Methods: Romaine lettuce leaves were cut into 5 x 5 cm^2 pieces and spot-inoculated with approximately 10^7 PFU/g of MNV-1 and left to dry in a biohazard cabinet to allow for viral attachment. The leaves were washed with a number of sanitizers including chlorine and peroxyacetic acid (POAA) at concentrations between 20 to 100 ppm with and without application of HPU for 60 s. After washing, leaves were transferred into a stomach bag containing 20 ml of phosphate buffered saline with 0.05M sodium thiosulfate to neutralize the sanitizer. Viruses were extracted and recovered from the samples and quantified by viral plaque assay.

Results: Four sanitizers were tested and chlorine and POAA had antiviral properties. POAA was effective in inactivating up to 4.6 log of MNV-1 on romaine lettuce. At concentrations of 20, 40, 60, 80 and 100 ppm, the reduction of MNV-1 was 2.1±0.2, 2.9±0.3, 3.2±0.3 3.7±0.2 and 4.2±0.3 log PFU/g, respectively. With 30 and 80 ppm chlorine, 2.0±0.7 and 2.3±0.3 log PFU/g reduction was observed, respectively. The use of HPU over the same concentrations provided additional 0.5~1 log reductions of MNV-1.

Significance: The results showed that POAA was an effective sanitizer for virus and HPU could enhance the performance of POAA on MNV-1 on fresh romaine lettuce during washing.

P1-44 Development of Hot Water Treatment for Inactivation of *Salmonella enterica* on Mung Bean Seeds

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Introduction: Foodborne outbreaks have been associated with the consumption of fresh sprouted beans. The use of a kill step on the seeds prior to sprouting step would enhance the safety of fresh sprouts.

Purpose: The objective of this work was to evaluate the effectiveness of hot water treatment in eliminating *Salmonella enterica* on artificially inoculated mung bean seeds.

Methods: Mung bean seeds were artificially inoculated with a cocktail of four *Salmonella* strains and stored at 4°C for 24 h. The effectiveness of inactivating *Salmonella* cells on the inoculated seeds using hot water at 75-90°C for up to 120 s was investigated. The effect of hot water treatment on seed sprouting and recovery of *Salmonella* cells from sprouted seeds were also investigated.

Results: Although, no viable *Salmonella* was recovered from seeds treated in hot water at 90°C for up to120 s, these treated seeds failed to germinate. *Salmonella* was not recovered from seeds treated at 80°C for 100 and 120 s, and from the sprouted seeds. Seed germination ability of treated seeds at 80°C for 100 and 120 s was reduced by 1-4% as compared to the non-treated controls.

Significance: The data presented here suggested that hot water treatments were capable of penetrating and inactivating cells which are attached to inaccessible sites and/or are within biofilms on the seed surface. The results presented here suggest that hot water treatment would be a viable process for enhancing the safety of fresh sprouts.

P1-45 Efficacy of *Lactobacillus plantarum* on the Reduction of *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella* spp. on Fresh-cut Granny Smith Apple Slices

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❖ ***Developing Scientist Competitor***

Introduction: Minimally processed fruits are often peeled, cut, or diced which compromise the fruits' natural protective barriers, exposing a nutrient-rich medium and providing an ideal environment for the growth of microorganisms, including foodborne pathogens. Recent outbreaks associated with minimally processed fruits indicate additional measures are needed to improve product safety. Application of biological control agents postharvest could provide an additional hurdle to the growth of foodborne pathogens.

Purpose: The purpose of this study was to evaluate the efficacy of *Lactobacillus plantarum* against *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella* spp. on fresh-cut apple slices.

Methods: Apple slices inoculated with *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* spp. (10^4 CFU/g) were treated with *L. plantarum* (10^7 CFU/g). Apples were stored at 4°C and 20°C for seven and five days, respectively. Population means and standard deviations of treatment groups were determined. One way analysis of variance (ANOVA) and Tukey pairwise comparison were used to determine if there was a difference between treatments (α = 0.05).

Results: Populations of *L. plantarum* were maintained throughout storage at a concentration of 7.0 ± 0.4 log CFU/g at 4°C and 20°C. Populations of *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* spp. inoculated alone were not significantly different than populations treated with *L. plantarum* at 4°C. Populations of *E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* spp. co-inoculated with *L. plantarum* were approximately 1.9 log CFU/g, 3.2 log CFU/g, and 0.5 log CFU/g less than populations inoculated alone after one, three, and five days of storage at 20°C.

Significance: Application of *L. plantarum* on fresh-cut apples could potentially provide a hurdle to the growth of foodborne pathogens.

P1-46 The Effect of Postharvest Practices on *Listeria monocytogenes* Contamination and Survival in Apple Fruit

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Introduction: Apples emerged as a new concern for *Listeria monocytogenes* contamination in the past few years. The 2012 *Missa Bay LLC*, 2015 *Northstar Produce Inc*. and *Del Monte Fresh Produce N.A. Inc.* multistate recalls of whole fresh apples due to contamination with *L. monocytogenes* clearly indicates the need for investigating the origin, spread, and persistence of this pathogen on pome fruits.

Purpose: Evaluate the effect of current postharvest practices used at commercial fruit packing facilities on the contamination and persistence of *L. monocytogenes* in intact apples.

Methods: Freshly harvested Granny Smith apples and two *L. monocytogenes* outbreak strains linked to caramel apples were evaluated. To imitate apple contamination during dump-tank washing, fruits were immersed into contaminated water (6 log CFU/ml) to various depths (surface, 1 and 2 feet) for 20 min. To track water uptake by apples, Acid Blue 9 dye (100 mg/ml) was dissolved in the water. Seven days after inoculation, detection and enumeration of *L. monocytogenes* internalized into different areas of the apple fruit core was conducted following FDA-BAM protocols.

Results: Dye infiltration into apple core was observed from both calix and stem ends of the fruit. Under experimental conditions *L. monocytogenes* internalized into inner calix and stem areas of the apple core during the dump tank washing. One week after inoculation bacterial populations in inner core ranged from 1 to 3 log CFU/g. The incidence of *L. monocytogenes* infiltration significantly ($P \leq 0.5$) varied among treatments and was 52.1, 65.0 and 81.6% for the surface, 1 and 2 feet for immersed apples, respectively. No difference in *L. monocytogenes* infiltration incidence was observed between calix and stem inner sinuses of Granny Smith apples.

Significance: Identification of postharvest practices potentially facilitating contamination and survival of *L. monocytogenes* in apples will permit prevention of future recalls and outbreaks of foodborne listeriosis associated with consumption of pome fruits.

P1-47 Effect of Weed Levels on Microbial Die-off Rate on Watermelon Surface in an Agricultural Setting

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Introduction: The Food Safety Modernization Act (FSMA) produce safety rule has placed emphasis on considering microbial die-off rate to evaluate the food safety risk associated with fresh produce.

Purpose: This study investigated the effect of weeds level on the survival of microorganisms on watermelon surface in an on-farm experiment.

Methods: Replicated watermelon plots (18 plots, 3 plots per treatment, plot size 12'x30', 30 plants per plot) were treated with herbicides (Strategy, Command 3ME, Strategy + Sinbar, Valor and Sinbar and an untreated check). Watermelon samples (n=80 total) were examined for the presence *E. coli* O157 and *Salmonella* spp. using immunomagnetic separation technique and quantified for total coliforms and generic *E. coli* using Quanti-Tray and petrifilm. Additionally, survival of generic *E. coli* inoculated on watermelon discs (20 cm^2 surface area) (6.24 log CFU/cm^2) under different weed levels was examined by placing them in each plot up to five days.

Results: Pathogens were not detected from the watermelon samples. Total coliform levels were higher (3.91 log CFU/cm^2) on watermelon harvested from high weed plots than from other plots (3.67 log CFU/cm^2). Watermelons harvested from medium weed areas had higher generic *E.coli* levels (1.46 log MPN/watermelon) than high weed (1.23 log MPN/watermelon) and low weed (1 log MNP/watermelon) areas. A significant (P<0.05) die-off of generic *E. coli* was observed within 12 h on watermelon disc from all weed level plots (>5 log CFU/cm^2 in no weed plots and, 0.95-1.14 log CFU/cm^2 from other plots); however the level was increased after 60 h. After 108 h, generic *E. coli* levels on watermelon discs from low weed plots (3.28 log CFU/cm^2) were significantly (P<0.05) lower as compare to medium and high weed plots (6.12 – 6.40 log CFU/cm^2).

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Significance: Our results suggest higher microbial die-off rate on produce surfaces exposed to sunlight radiation.

P1-48 Antimicrobial Effectiveness of Coating Solutions Containing Chitosan, Lauric Arginate Ester and Allyl Isothiocyanate against *E. coli* O157:H7 and *Salmonella* spp. on Strawberries

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Introduction: An outbreak of hemorrhagic colitis in 2011 was associated with contaminated strawberries, which resulted in two deaths. There is a need for a preservation technique that reduces pathogenic contaminants on fresh strawberries without compromising quality. In our previous studies, lauric arginate ester (LAE), allyl isothiocyanate (AIT) and organic acids were used for antimicrobial coatings and films, and were effective in inhibiting foodborne pathogens in ready-to-eat meat, shrimp, and cantaloupe. However, optimization of these coating formulations for strawberries has not been further investigated.

Purpose: This study was conducted to identify different coating formulations for inactivating *E. coli* O157:H7 and *Salmonella* spp. from artificially-inoculated strawberries.

Methods: Ten edible coating formulations were developed, which consisted of chitosan (1%), AIT (0, 1, 2 %), LAE (0, 1, 2%), organic acids (levulinic acid + lactic acid; 0.5, 1%), and an emulsifier (0.167, 0.5%). The coating solutions were high pressure homogenized and directly coated on the surface of strawberries inoculated with a six-strain composite of *E. coli* O157:H7 (ATCC 43895, ATCC 43894, and strain C9490) and *Salmonella* (Newport H 1275, St. Paul 02-517-1, and Stanley H0558). Strawberry samples were placed in PET boxes and stored at 4°C. Survival of *E. coli* O 157:H7 and *Salmonella* were determined after 1, 2, and 5 days of storage.

Results: All coatings containing AIT and LAE significantly (P<0.05) inactivated *E. coli* O157:H7 and *Salmonella* on the surface of strawberries stored at 4°C for 5 days. When the same concentrations were used, AIT showed significantly stronger antimicrobial activity than LAE. The coatings reduced pathogens by 1.5, 2.0, and 4.0 log CFU/strawberry in 1, 2 and 5 days, respectively. Higher concentrations of acids (1% vs. 0.5%) or emulsifiers (0.5% vs. 0.167%) did not show significant difference in antimicrobial activity.

Significance: Results from this study provide some options for decontaminating strawberries prior to serving to customers.

P1-49 Opposite Inactivation Responses to Process Temperature by Virus Surrogates MNV-1 and MS2 during High Hydrostatic Pressure Processing of Contaminated Fruit Puree and Juices

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Introduction: High hydrostatic pressure processing (HPP) is known to inactivate pathogens and retain fruit qualities via the controls of process temperature, pressure and time.

Purpose: This study evaluated the effectiveness of HPP holding-temperature (0 to 38℃) on inactivating virus surrogates of MNV-1 and MS2 in pomegranate juice, strawberry juice and puree.

Methods: Murine norovirus (MNV) as a norovirus (NoV) surrogate was inoculated into strawberry or pomegranate juice, and adjusted for initial temperature to reach the process holding-temperature of 10, 20, or 30℃, along with 300 MPa for 3 min for virus inactivation comparison. MS2 coliphage, a relatively HPP-resistant surrogate, was inoculated into strawberry puree and then treated with 600 MPa, 3 min, and holding temperatures of 15 to 38℃.

Results: A regression formula was derived (n=22) for MNV inactivation in juices: Y (log-reduction) = - 0.08 * X (holding-temp.) + 2.6 logs, r^2= 0.97. This predicted a 2.6-log reduction of MNV in both juices at HPP 0℃-holding, with MNV inactivation being inversely proportional to temperature increase. When the process temperature and pressure were evaluated individually, the 5-log reduction process for HPP-sensitive MNV in strawberry puree was predicted to be 424 MPa for 3 min at 20℃ holding. On the contrary, surrogate MS2 coliphage inactivation was greater as HPP holding temperature increased (15 to 38℃), all under 600 MPa for 3 min. The increased kill is presumably similar for hepatitis A virus (HAV); but the HPP holding-temperature was not correlated to the reduction level of relatively resistant MS2 in strawberry puree. Overall an increase in process temperature does not resulted in the same killing effect especially for NoV and HAV, although elevated pressure and time generally increase the kill.

Significance: This research provides possible means to manipulate/control HPP process temperature for effective inactivation of different foodborne viruses in fruit juices and puree.

P1-50 The Use of a Commercial Naturally-occurring Citrus-based Sanitizer to Prevent Cross-contamination of *Listeria monocytogenes* on the Surface of Organic Cantaloupes

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❖ ***Developing Scientist Competitor***

Introduction: The microbiological safety of cantaloupes has been a major concern since a 2011 illness outbreak caused by *Listeria monocytogenes*. An effective sanitizer is needed to reduce microbial loads on cantaloupe surfaces, prevent cross-contamination during handling, and function in the presence of organic loads. Additionally, methods need to be developed that meet the stringent requirements for organic growers.

Purpose: The purpose of this study was to evaluate the antimicrobial efficacy of a novel commercial naturally-occurring citrus-based sanitizer for preventing cross-contamination of USDA-certified organic cantaloupes during post-harvest washing.

Methods: A 5 strain cocktail of *Listeria monocytogenes* (LM) resistant to nalidixic acid (NAR) was prepared. A microbroth dilution assay was used to determine the minimum inhibitory concentration (MIC) of the commercial sanitizer (CX; Citrox, Middlesborough, UK) against LM. Wild type (WT) and NAR strains were compared to confirm resistance to CX. Cantaloupe samples were inoculated with LM and placed in solutions of 0%, 0.5%, 0.75% CX (v/v), or 200-ppm chlorine, with or without 2.0% organic load (OL). To determine if cross-contamination occurred in treatment liquids, un-inoculated cantaloupe was introduced to the same solutions after the inoculated samples. After sanitizing, all samples were removed and hand-massaged. The rinsate was plated TSA + nalidixic acid + 0.6% Yeast Extract to enumerate survivors.

Results: The MIC for all WT and NAR LM was 0.5% CX. For the initial sampling, 0.5% CX and chlorine solutions reduced survival of LM by 1 log (90%). For un-inoculated produce, cross-contamination by LM was eliminated both by CX and chlorine compared to the control (ca. 6 to 7 log CFU/g). OL had a negative effect on CX and chlorine inactivation, except for 0.75% CX.

Significance: Results indicate that 0.75% CX had efficacy in eliminating cross-contamination on organic produce even in the presence of OL in a model wash system.

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P1-51 Estimation on the Consumption Patterns of Livestock and Processed Livestock Products in Korea

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Introduction: An estimation of food consumption details, such as portion size and frequency of consumption, is needed for exposure assessment in microbiological risk assessment.

Purpose: This study investigated the amount and frequency of 50 kinds of consumed livestock products and analyzed the key factors that affect the consumption and purchase of livestock and processed livestock products.

Methods: A quantitative survey was performed by trained interviewers in face-to-face interviews, with 1,500 adults aged over 18, who were randomly selected from six major provinces in Korea. Respondents received a picture of one serving size for each of the 50 livestock products, including meats, processed meat products, dairy products and processed egg products. A T-test and general linear model (GLM) were carried out using SPSS statistics.

Results: The most frequently consumed food was milk (2.6 times/week), followed by pork (1.4 times), liquid yogurt (1.3 times) and rolled omelet (1.2 times). In the case of the amount of consumption, people living in the city consumed meat (beef, pork, chicken and duck) 1.5 times more than those living in the village. When people eat meat, they consume twice of one serving size. The most important factor affecting consumption of livestock products was the residence area. As customers perceive the risk of food poisoning from raw meat, the purchase and intake decreased (P<0.001). The most preferred cooking method was roasting, regardless of the type of meat, but the second preference for cooking was significantly affected by the type of meat (P<0.001): stir-fried pork, beef with seasoning, fried-chicken and duck soup.

Significance: The data from this study can be used for risk assessment of livestock and processed livestock products and education for safe consumption and purchase of livestock products.

P1-52 Prevalence of *Salmonella* spp. in Retail Chicken Meat: A Multistate Study from Mexico

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Introduction: *Salmonella* is an important health problem around the world, and meat is considered a main source of contamination. Chicken meat has been linked to numerous *Salmonella* outbreaks in USA and Europe. Thus, it is fundamental for health authorities and consumers to know the prevalence of this pathogen in chicken meat from retail markets. Unfortunately, this information is limited or lacking in Mexico.

Purpose: The objective of the present study was to evaluate the prevalence of *Salmonella* spp. in retail chicken meat from public markets and supermarkets from different geographical regions of Mexico.

Methods: A total of 1,293 chicken necks from public markets and supermarkets were obtained from six Mexican states. The microbiological analysis was carried out following methodology described by the European Food Safety Authority. Briefly, 25 g of neck skin (obtained from 3 necks) were pooled and cultured in buffered peptone water, tetrathionate and Rappaport-Vassiliadis broth and Xylose-Lysine-Deoxycholate agar supplemented with novobiocin (0.001%). Confirmation of presumptive *Salmonella* colonies was performed by PCR targeting the *16s rRNA* and *invA* genes.

Results: The average prevalence of *Salmonella* spp. in Mexico was 5.8%. Prevalence in supermarkets was 7.9% and 4.2% in public markets (P > 0.05).

Significance: The present study provides the first multistate data describing the prevalence of *Salmonella* spp. in retail chicken meat. The prevalence observed in Mexico concurs with levels reported in USA and Europe. Thus, as in many other countries, chicken meat in Mexico should be considered an important health threat.

P1-53 Antimicrobial Resistance of *Salmonella enterica* from Chickens in South Korea

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Introduction: *Salmonella enterica (S. enterica)* infections by poultry products has been a major food-borne illness even though only several serotypes cause most human infections. For control, antimicrobial drugs have been widely used and the emergence of multidrug resistance especially to fluoroquinolones and third-generation cephalosporins associated with poultry is also an increasing threat to public health in South Korea.

Purpose: In the present study, we examined antimicrobial resistance of recent isolates of *S. enterica* from chickens and environments for the control of *Salmonella* infection in chicken.

Methods: *S. enterica* isolates (n=104) from chickens and environments of chicken farms and slaughter houses in South Korea in 2015 were serotyped and tested for an antimicrobial susceptibility by determining minimum inhibitory concentrations(MICs) to 15 antimicrobials using customized panels (Sensititre; Trek Diagnostics, Westlake, OH, USA).

Results: Thirteen serovars of *S. enterica* were identified in the isolates except 2 unidentified isolates. A total of 104 isolates of *S. enterica* from chickens and environments showed 32 different antimicrobial resistance pattern, respectively. Most isolates of *S. enterica* (94 /104; 90.4%) were resistant to nalidixic acid and 61.5% (64/104) of the isolates showed resistance to at least five antimicrobials. Moreover, the resistance rate to third-generation cephalosporin (ceftiofur) and fluoroquinolone was 17.3% (18/104) and 2.9% (3/104) in *S. enterica* isolate, respectively

Significance: These data showed high frequency of multi-drug resistance (MDR) and also resistance to fluoroquinolones and third-generation cephalosporins in *S. enterica* isolates from chickens and environments in South Korea. Appropriate intervention strategies is needed for control of *Salmonella* infections and reducing antimicrobial resistance in chickens.

P1-54 Prevalence and Antibiotic Susceptibility of Pathogenic *Escherichia coli* Recovered from Pig and Cattle Slaughterhouses

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Introduction: Pathogenic *Escherichia coli* (PEC) was considered important food-borne pathogens, and recognized as a significant public health problem. In most case, contaminated animal products may be responsible for PEC infections in humans. The animal carrying and shedding PEC and other pathogenic microorganisms in slaughterhouses is the main source of contamination.

Purpose: The aim of this study was to investigate the isolation rate of pathogenic *Escherichia coli* from pig and cattle slaughterhouses.

Methods: The PEC was determined in pig carcasses (n = 245), cattle carcasses (n = 210), pig carcasses chilling room (n = 98), and cattle carcasses chilling room (n = 84), collected from 50 slaughterhouses in South Korea. In order to detect a virulence factor, we selected the target genes: *stx1* and

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stx2 for STEC; *eaeA* for EPEC; *ipaH* for EIEC; *elt*, *estp*, and *esth* for ETEC; *aggR* for EAEC. The antibiotic susceptibility of PEC isolates was determined by the disk diffusion method according to NCCLS

Results: A total of 14 PEC isolates were isolated from 12 slaughterhouses; 2 of 245 of pig carcasses (0.82%), 8 of 210 of cattle carcasses (3.81%), 2 of 98 of pig carcasses chilling room samples (2.04%), and 2 of 84 of cattle carcasses chilling room samples (2.38%). Virulence genes of at least one PEC pathogroup was detected in 14 (2.20%) of the 637 samples, with 7 (1.10%) being positive for virulence genes of STEC, 6 (0.94%) of EPEC and 1(0.16%) of ETEC. The antibiotic resistance observed was with tetracycline, streptomycin and chloramphenicol (14.29%) followed by ciprofloxacin (7.14%).

Significance: Pig and cattle carcasses and their storage condition should be monitored to prevent pathogenic *Escherichia coli*. The origin of infected slaughter animals should be identified and direct and cross-contamination of carcasses should be avoided by adhering to HACCP principles in association with good hygiene procedures (GHP).

P1-55 Occurrence and Antimicrobial Resistance of *Enterobacteriaceae* in Shell Eggs from Small-scale Poultry Farms and Farmers' Markets

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Introduction: Poultry eggs are a nutritious, low-cost source of protein, and are extensively consumed around the world. However, *Enterobacteriaceae* including *Salmonella* and *Escherichia coli* have been isolated from shell eggs and their contents. Members of *Enterobacteriaceae* family are becoming resistant to major antibiotics used in human medicine.

Purpose: The purpose of this study was to investigate the prevalence and antimicrobial resistance of *Enterobacteriaceae* recovered from shell eggs, collected at small-scale poultry farms and farmers' markets.

Methods: Shell eggs (n=504) were collected and two eggs were constituted to make 252 composite samples. *Enterobacteriaceae* were enumerated by using violet red bile glucose agar plates and identified through biochemical tests and PCR. Antimicrobial susceptibility to 13 antimicrobial agents was determined by Kirby-Bauer's disc diffusion method and results interpreted by CLSI interpretative values.

Results: Of a total of 252 composite eggs (shells, egg contents), 30 (11.9%), 23 (9.1%), and 29 (11.5%) egg shells alone and 13 (5.2%), 20 (7.9%), and 12 (4.8%) egg contents alone were positive for *Escherichia coli, Enterobacter* spp, and *Serratia* spp, respectively. The prevalence of *Salmonella* was 3.6% on the egg shells and none in the egg contents. *Enterobacteriaceae* level on shell eggs (4.4±2.0 log CFU/egg shell) was significantly higher ($P \leq 0.05$) in shell eggs from poultry farms than found on egg shells from farmers' market (2.1±1.3 log CFU/egg shell). Out of the 134 *Enterobacteriaceae* isolates tested, resistance to erythromycin (80.6%), ampicillin (61.9%), tetracycline (50.7 %), and streptomycin (45.5 %) was observed.

Significance: Our results suggest that shell eggs from small-scale poultry farms and farmers' markets may be contaminated with antibiotic resistant foodborne and commensal bacteria. These observations also underline the need to educate the public on the risks from mishandling raw eggs and consumption of undercooked eggs or egg products.

P1-56 Withdrawn

P1-57 *Escherichia coli* O157:H7 and Non-O157 Shiga Toxin-producing E. coli (STEC) in Beef Manufacturing Trimmings Samples (MT60 Sampling Project) Analyzed by the Food Safety and Inspection Service from Fiscal Years 2012 to 2015

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Introduction: The Food Safety and Inspection Service (FSIS) analyzed data from its routine regulatory testing project for STEC (both O157:H7 and non-O157) in beef manufacturing trimmings (MT60 sampling). In June 2012, FSIS began testing beef manufacturing trimmings for the 7 STEC (O157:H7, O26, O45, O103, O111, O121 and O145) that it regulates as adulterants.

Purpose: To analyze MT60 beef manufacturing trimmings sampling data after initiating non-O157 STEC testing.

Methods: FSIS analyzed MT60 sample results from June 2012 to September 2015.*

Results: From June 2012 through September 2015, FSIS collected and tested 10,025 MT60 samples from 540 establishments for *E. coli* O157:H7 and 9,338 samples from 525 establishments for non-O157 STEC, and the overall percent positives were 0.32% and 0.74%, respectively. For Fiscal Years 2013-2015 the positives ranged from 0.13 to 0.50% for *E. coli* O157:H7 and 0.16 to 0.49% for non-O157 STEC. The most frequently identified non-O157 STEC was O103, followed by O26 and O111. Very few samples were positive for more than one O-group isolate. On an establishment basis, 5.2% and 9.0% establishments had at least one positive for O157:H7 and non-O157 respectively. Most of the STEC-positive samples were obtained from small or very small establishments based on HACCP size categories. Percent positives were higher in trim samples produced from veal (4.16%) than from adult cattle (0.45%). Seasonal variation was seen in the presence of STEC with higher percent positives recorded from June to September. Finally, percent positives were higher in Northeast, North Central and Southeast regions than in the Southwest or West.

Significance: This analysis informs policy development. More data are needed to determine whether these trends continue and to determine whether there are additional factors to consider.

*FSIS acknowledges the contributions of its field inspectors and lab personnel in sample collection and testing.

P1-58 Prevalence of Rotavirus and Porcine Enteric Calicivirus at Various Stages of Pork Carcass Processing

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Introduction: Porcine enteric calicivirus (PEC) and rotavirus (RV) infections are common in swine and raises concerns about the potential for zoonotic transmission through undercooked meat products. Enteric viruses can potentially contaminate carcasses during meat processing operations. There is a lack of information on the prevalence and control of enteric viruses in the pork processing chain.

Purpose: The study compared the incidence and levels of contamination of hog carcasses with F- coliphages (FC), RV and PEC at different stages of the dressing process.

Methods: One hundred swab samples (100 cm^2) were collected from random sites on hog carcasses at 4 different stages of the dressing process and from meat cuts on 10 separate occasions over the span of a year from 2 pork processing plants (1,000 samples in total, 500/plant). Numbers of viable FC were determined by plaque assay and the numbers of genome copies (GC) of RV and PEC were determined by qRT-PCR.

Results: FC, RV, and PEC were detected in 100%, 100%, and 18% of samples, respectively, after bleeding for plant 1 and in 100%, 98%, and 36% of samples, respectively, after bleeding for plant 2. After evisceration, FC, RV, and PEC were detected in 63%, 21% and 3% of samples, respectively, for plant 1 and in 25%, 1%, and 0% of samples, respectively for plant 2. FC, RV, and PEC were detected on 21%, 1%, and 5% of pork cuts, respectively, for plant 1 and on 13%, 0%, and 0% of pork cuts, respectively, for plant 2.

Significance: The prevalence of PEC and RV on pork is progressively reduced along the pork processing chain but the viruses are not completely eliminated. The simultaneous detection of viable FC suggests that consumers could be at risk when consuming undercooked meat contaminated with pathogenic enteric viruses.

P1-59 Prevalence and Pathogenic Potential of *Escherichia coli* O157:H7 Isolates Recovered from Veal Products Purchased at Retail Establishments in the Mid-Atlantic Region of the United States

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Introduction: *Escherichia coli* O157:H7 (ECOH) is a significant public health concern in many countries and associated with a broad spectrum of infections which range from mild diarrhea to hemorrhagic colitis and hemolytic uremic syndrome. Recently, recalls of several thousand pounds of ECOH contaminated retail veal from across the U.S. have increased interest in the prevalence and pathogenic potential of this pathogen.

Purpose: Investigate the prevalence and pathogenic potential of ECOH isolated from retail veal in four states in the Mid-Atlantic region of the U.S.

Methods: Ground veal (*n*=372) and veal cutlets (*n*=250) were purchased at retail stores in Delaware (*n*=144), Maryland (*n*=155), Virginia (*n*=142) and Pennsylvania (*n*=181) over a 12-month period spanning 2014 and 2015. All samples were enriched and then screened for the presence of ECOH using the BAX System Real-Time PCR. The BAX-positive cultures were further analyzed by a culture-based protocol involving immuno-magnetic separation (IMS) and plating on Rainbow Agar (RA). Isolates were analyzed by PCR for the presence of stx_1, stx_2, intimin (*eae),* EHEC-hemolysin (*ehx*), O157 (*rfb*E), and H7 (*fli*C) genes. Subtypes of stx_1 and stx_2were identified by nucleotide sequencing.

Results: Three of 622 samples (0.2%) tested positive for ECOH by BAX: one each from MD (ground veal), DE (ground veal), and PA (veal cutlets). However, only the sample from DE tested positive by both BAX and IMS. Isolates (*n*=10) recovered from the DE sample tested positive by BAX for all 6 genes screened, whereas MD isolates (*n*=10) were positive for only the O157 gene. Isolates (*n*=10) from PA were negative for all genes. All O157 isolates were positive for either stx_{1a} or stx_{2a}.

Significance: These results suggest the prevalence of ECOH in retail veal is quite low and shed insight on the pathogenic potential of ECOH recovered from veal at retail establishments.

P1-60 Microbiological Profile of Different Steps during Pig Slaughter

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Introduction: During the pig slaughter process there are several steps where microbial levels on carcasses can change. Therefore, to know the points where contamination increases or decreases is strategically important to establish measures of control to reduce microbiological risks.

Purpose: Identify the contamination by *Salmonella*, *Listeria monocytogenes* and indicator microorganisms in main steps along the pig slaughter in an industry located in south of Brazil to map the critical points of microbiological contamination.

Methods: Samples of pig carcasses were collected after different steps of the slaughter process (bleeding, scalding, dehairing, singeing, washing after toilet, evisceration, inspection, final washing and chilling), (*n* = 130). Detection of *Salmonella* was conducted using PCR BAX System and *L. monocytogenes* by VIDAS. Aerobic mesophilic (AM), *E. coli* (EC), total coliforms (TC) and *Enterobacteriaceae*(EB) were analyzed using 3M Petrifilm. The result was statistically evaluated through the ANOVA and Tukey test.

Results: *Salmonella* was present in 26.6% of carcasses analyzed in the initial stage and in 1.11% in the last step. Detection of *Salmonella* was heterogeneous, indicating that the presence of bacteria is dependent of several factors. *Listeria monocytogenes* was only detected in carcasses from the final wash (21.1%) and in chilling (8.9%). For indicator microorganisms statistical difference ($P > 0.05$, Tukey test) was observed between same steps for all the microorganisms tested. From beginning to the end of the process, reduction of 1.6 for AM, 0.7 for EB, 0.8 for EC and 0.7 log CFU/g for TC was observed.

Significance: The study was used to evaluate the microbiological performance of a pig slaughter industry in Brazil and establish strategies to decrease microbiological contamination.

P1-61 Correlation between Quality and Hygiene Indicator Microorganisms with Pathogens in a Pig Slaughter Process

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Introduction: The microbiological control of a slaughter process should evaluate the prevalence of pathogens and levels of quality and hygiene indicators microorganisms. However, indicators microorganisms should provide information about the conditions of hygiene process and be correlated with a possible presence of pathogens.

Purpose: To evaluate the correlation between quality and hygiene indicators groups (mesophilic aerobic, *Enterobacteriaceae*, total coliforms and *Escherichia coli*) with the prevalence of pathogens (*Salmonella* spp. and *Listeria monocytogenes*) in a pig slaughter process.

Methods: Samples of pig carcasses were sampled in different steps of slaughter line using sterile sponges. Detection of *Salmonella* was conducted using PCR BAX System and *L. monocytogenes* by VIDAS. Aerobic mesophilic (AM), *E. coli* (EC), total coliforms (TC) and *Enterobacteriaceae*(EB) were analyzed using 3M Petrifilm. The results were analyzed by Pearson Correlation.

Results: Prevalence of *Salmonella* on carcasses was 15.8% and *Listeria monocytogenes* was 3.3%. Levels found on carcasses for AM, EB, TC and EC were 3.5 ± 0.9; 1.1 ± 0.7; 0.8 ± 0.6 and 0.5 ± 0.6 CFU/g, respectively. None of the four indicators groups tested had correlation with prevalence of *Salmonella* and *Listeria monocytogenes*.

Poster

Significance: Although it has not been possible to establish the correlation between a group of indicators with prevalence of pathogens, such as Salmonella and L. monocytogenes, the use of microorganisms indicators provide important information to improve quality microbiological of pig slaughter.

P1-62 The Control of *Salmonella* with Commercially Available Bacteriophage during Ground Chicken Processing

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❖ ***Developing Scientist Competitor***

Introduction: Salmonellosis is caused by ingesting *Salmonella* bacterial species. In the United States, it is the number one foodborne illness that results in hospitalization and/or death and causes approximately $365 million in direct annual medical costs. In 2012, the broiler chicken industry grossed $24.8 billion nationwide. Considering all this, poultry processors are looking towards alternative, non-antibiotic, methods of *Salmonella* control.

Purpose: The objective of this study was to ascertain the effectiveness of commercially available bacteriophage during ground chicken production based on the source of water used for sample dilution, *Salmonella* serotype, and treatment time.

Methods: *Salmonella*-free boneless-skinless chicken thighs from the processing line of a regional poultry producer were inoculated with 4.0 log CFU/cm^2 of either a cocktail of *Salmonella* isolated from ground chicken or a cocktail of laboratory *Salmonella* strains. Bacteriophages were applied to the chicken, per the manufacture's recommendations, using sterile tap or distilled water for 30 min and/or 8 h. *Salmonella* was recovered using standard plating method.

Results: Greater *Salmonella* reduction was observed when the bacteriophage was diluted in sterile tap water than sterile distilled water, achieving 0.23 log CFU/cm^2 and 0.39 log CFU/cm^2 reduction after 30 min, respectively. The ground chicken cocktail experienced 0.41 log CFU/cm^2 and 0.70 log CFU/cm^2 reductions after 30 min and 8 h, respectively (P<0.05). The isolates recovered from outbreaks showed reductions of 0.57 log CFU/cm^2 and 0.76 log CFU/cm^2 after 30 min and 8 h, respectively (P<0.05).

Significance: These results indicate that commercially available bacteriophages can significantly reduce *Salmonella* in ground chicken. However, reduction is dependent on water used to dilute the bacteriophage, the *Salmonella*'s susceptibility to the bacteriophage and treatment time, as indicated by the different levels of reduction.

P1-63 Pathogen Control Strategies Used by United States Meat Slaughter and Processing Establishments

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Introduction: Food safety technologies and practices by meat slaughter establishments are continuing to evolve with the ongoing goal of reducing pathogens that cause foodborne illness.

Purpose: The purpose of this study was to assess technological and food safety practices used in the meat slaughter and processing industry, and how these practices have changed since 2005.

Methods: We conducted a nationally representative survey of meat slaughter and processing establishments in 2015 (n=376, response rate of 66%). The questionnaire asked about operational and sanitation practices, microbiological testing practices, employee food safety training, and other food safety and marketing practices.

Results: In the slaughter and fabrication area, 93% of establishments use chemical sanitizers or hot water on food contact surface areas, and 66% of establishments use organic acid rinses on carcasses. After the slaughter and fabrication process, 72% of establishments process raw meat into further processed products. During this stage, nearly all establishments use chemical sanitizers or hot water to sanitize hand tools such as knives, increasing from 65% in 2005 to 98% in 2015. Approximately three-fourths (76%) of establishments conduct additional microbiological testing beyond what is required by regulation. Nearly all meat slaughter establishments train new hires on food safety procedures; unscheduled, on-the-job training was the most common type of training in 2005 and 2015. To ensure that written food safety plans are being followed, almost two-thirds (65%) of establishments are audited by independent, third-party auditors.

Significance: The results of the nationwide survey, and their comparison to a 2005 survey, indicate some food safety practices have increased over the past 10 years, while others remained the same or decreased. The study findings can identify areas in which improvements are needed, as well as guide regulatory policy making and inform regulatory impact analysis.

P1-64 Evaluation of *Salmonella* Biofilm Cell Transfer from Contact Surfaces to Beef Products

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Introduction: Meat contamination by *Salmonella enterica* is a serious food safety concern. One common transmission route that leads to cross contamination is bacteria transfer from biofilms on contact surfaces to meat products via direct contact. Many factors could affect biofilm transfer efficiency, including biofilm forming ability, food product composition and the type of contact surface materials. We evaluated the impact of these factors on *Salmonella* biofilm cell transfer from common contact surfaces to beef products. We also determined *Salmonella* prevalence after beef products contacting solid surfaces colonized by *Salmonella* biofilms.

Purpose: To evaluate the effects of biofilm forming ability, meat surface composition and contact surface materials on *Salmonella* biofilm cell transfer efficiency.

Methods: Four strains of *Salmonella* serotypes Anatum and Dublin were used to quantify enumerable amount of bacteria transferred from biofilms on solid surfaces to beef products after direct/consecutive contacts. The effect of direct contact with biofilm-colonized surface on *Salmonella* prevalence rate in beef products was determined after sample enrichment.

Results: Effective transfer of *Salmonella* cells from biofilms on solid surfaces to beef products via direct contact was observed. Biofilm forming potency had the most significant effect on transfer efficiency. Contact surface materials also affected the transfer as biofilms on stainless steel surface appeared to transfer more efficiently than those on polyvinyl chloride plastic surface (P<0.05). Conversely, meat surface composition (muscle or fat tissues) showed no significant effect on biofilm transfer efficiency. Importantly, after enrichment all beef samples that contacted *Salmonella* biofilms exhibited positive *Salmonella* prevalence, including samples with no enumerable transferred *Salmonella* cells.

Poster

Significance: These data indicate that *Salmonella* biofilms, even at low levels, still present a serious risk of causing cross-contamination, thus, highlight the importance of proper sanitization procedures in meat plants.

P1-65 Biofilm Formation by *Salmonella* Enteritidis in a Simulated Egg Processing Environment and Its Sensitivity to Chlorine and Hot Water Treatment

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Introduction: *Salmonella* Enteritidis outbreaks were usually associated with consumption of eggs. The ability of *Salmonella* Enteritidis to form biofilms on egg contact surface may serve as a source of cross-contamination, posing potential safety risks to consumers.

Purpose: This study aimed to investigate the biofilm formation by *Salmonella* Enteritidis in a simulated egg processing environment and its resistance to chlorine and hot water treatment.

Methods: Three *Salmonella* Enteritidis strains (ATCC 13076, 124 and 125) were individually inoculated into 0.1% peptone water (PW), 10% whole eggs (WE), 10% egg yolks (EY) and 10% egg whites (EW). Stainless steel coupons were immersed in the inoculated media and incubated at 25°C under a static condition. Biofilm densities were evaluated after 2, 4, and 7 days of incubation, and biofilms at day 2 or day 7 were treated by 200 ppm chlorine (pH 6.8) for 5 min or by hot water at 71.1°C for 30 s. Mean values were compared using ANOVA.

Results: Densities of *Salmonella* Enteritidis biofilms and their resistance to chlorine and hot water were affected by growth medium but not by strains and incubation time. Salmonella Enteritidis formed significantly (P < 0.05) denser biofilms in PW and EW (6.28 CFU/cm^2) than WE and EY (4.94 CFU/cm^2). However, biofilms formed in PW were less resistant to chlorine treatment than those formed in WE, EY, and EW, with average log reductions of 6.41, 2.28, 0.63 and 0.95 CFU/cm^2, respectively. All biofilm cells were very sensitive to hot water treatment, which reduced the cell populations by 4.30-6.39 log.

Significance: The study provides information on the biofilm forming abilities of *S.* Enteritidis in a simulated egg processing environment and the effectiveness of chlorine and hot water treatment against *Salmonella* Enteritidis biofilms, which may aid in the development of better sanitation strategies.

P1-66 Antimicrobial Performance on Pathogen Surrogates and Natural Flora Populations of Chicken Parts and Effect during Product Shelf Life

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Introduction: There has been an enhanced regulatory focus on *Salmonella* prevalence on poultry parts.

Purpose: This experiment examined *Salmonella* surrogate survival on poultry parts after an antimicrobial application and the growth of aerobic and anaerobic spoilage organisms after application.

Methods: Boneless, skinless chicken breasts and thighs were inoculated with rifampicin-resistant *Escherichia coli* (5-strains; 5.5 log CFU/cm^2) and exposed to water, peroxyacetic acid (PAA; 850 ppm, 400 ppm), and a lactic and citric acid blend (LCA; 2.25%). Solutions were applied in a commercial spray cabinet (1.4 bar, 2 l/min, and 0.9 cm/s) containing a single top and bottom spray bar. Uninoculated chicken parts were similarly exposed to antimicrobials, packaged in a modified atmosphere (64% oxygen, 31% carbon dioxide), and stored at 4.0°C for up to 17 days until sampled for oxygen and carbon dioxide content of package and total aerobic bacteria (TAB), *Pseudomonas* spp., and lactic acid bacteria (LAB) populations of product. Data (log CFU/cm^2, 2 replicates/3 samples per replicate) were analyzed using ANOVA in SAS.

Results: All antimicrobial solutions were effective in reducing (P<0.05) inoculated *E. coli* and TAB, *Pseudomonas* spp., and LAB populations naturally occurring on chicken breasts (0.3-2.3 log CFU/cm^2) and thighs (0.2-1.4 log CFU/cm^2). The order of efficacy for the solutions on both chicken breasts and thighs was 850 ppm PAA > 400 ppm PAA > 2.25% BLC. Differences (P<0.05) in bacterial recovered from poultry parts treated with antimicrobials and those untreated were maintained throughout the 17-day shelf life. LAB growth was more pronounced from day 0 to day 17 (1.0-5.6 log CFU/cm^2) than *Pseudomonas* spp. growth (1.1-1.4 log CFU/cm^2), which can be attributed to the oxygen and carbon dioxide content of packaging.

Significance: These data indicate an antimicrobial application may be effective in reducing *Salmonella* populations on chicken parts and improve product bacterial quality during shelf life.

P1-67 Evaluation of Antimicrobial Effects on Pathogen Reduction on Chicken Carcass during First Processing

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Introduction: *Salmonella* and *Campylobacter* are representative foodborne pathogens causing foodborne illness in humans referred to as salmonellosis and campylobacteriosis, respectively. Several antimicrobials have been utilized to reduce pathogenic bacteria contamination during first chicken carcass processing plant.

Purpose: The purpose of this study was to evaluate the antimicrobial efficacy of peracetic acid (PAA) and Amplon (a commercial blend of sulfuric acid and sodium sulfate) for *Salmonella* and *Campylobacter* reduction on chicken carcasses during first processing.

Methods: Chicken carcass rinsates (10 birds) were collected from eight different stages (group A to H): Group A) Before depilation, B) post-pick before Amplon spray, C) post-pick after Amplon spray, D) post evisceration before simulated on-line reprocessing (OLR) with Amplon, E) post evisceration and after simulated OLR, F) after primary chilling with PAA, G) after post-chilling with Amplon, and H) after post-chilling with PAA.

Results: The populations of *Campylobacter* were significantly reduced by antimicrobial applications. Amplon spray, simulated OLR, post-chilling with Amplon and PAA exhibited 3.25, 1.15, 1.52, and 2.22 log CFU/chicken reduction of *Campylobacter* (P < 0.05), respectively, while there was no *Campylobacter* reduction in primary chilling with PAA (P ≥ 0.05). Similar to *Campylobacter* results, antimicrobials applications including Amplon spray, post-chilling with Amplon and PAA also reduced the prevalence of *Salmonella*. *Salmonella* were isolated from 3 control (group A), 10 before Amplon spray (B), 2 after Amplon spray (C), 5 before simulated OLR (D), 4 after simulated OLR (E), 4 after primary chilling (F), and 2 after post-chilling with Amplon (G) chicken carcasses. No *Salmonella* was detected in a group H (after post-chilling with PAA).

Significance: These decontamination methods could be utilized as a part of the antimicrobial application in poultry industry to ensure the microbiological safety of poultry products.

Poster

P1-68 Study the Effectiveness of Trisodium Phosphate and Citric Acid to Reduce Microbial Load in Beef and Poultry and Ionizing Irradiation to Eliminate Foodborne Diseases

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Introduction: *Escherichia coli* O157:H7 and *Salmonella* Enteritidis are the most important foodborne pathogens that cause of diseases transmitted through meat and meat products.

Purpose: The objective of this study was to use trisodium phosphate and citric acid combination with irradiation to reduce the microbial load and to eliminate pathogens populations in beef and chicken meat.

Methods: Beef and chicken meat samples were dipped in 10% solution of trisodium phosphate and 2% citric acid for 1 min. Beef was inoculated with *E. coli* O157:H7 and chicken with *Salmonella* Enteritidis and both dipped in 10% solution of trisodium phosphate, and 2% citric acid for 1 min. After that the samples were treated by gamma irradiation in the range of 1 to 5 kGy.

Results: The reduction of aerobic total counts in beef and chicken meat dipped in 10% of trisodium phosphate decreased by 3.2 and 2.5 logs CFU/g, respectively. Also, aerobic total counts in beef and chicken samples dipped in 2% citric acid were 1.8 and 1.5 logs CFU/g, respectively. The reduction of *E. coli* 0157:H7 in beef and *Salmonella* Enteritidis in chicken dipped in 10% of trisodium phosphate decreased by 2.6 and 1.9 logs CFU/g, respectively. The reduction of *E. coli* 0157:H7 in beef meat and *Salmonella* Enteritidis in chicken meat dipped in 2% of citric acid decreased by 2.0 and 1.5 log CFU/g, respectively. An irradiation dose of 1.5 kGy in combination with trisodium phosphate and citric acid can be significantly eliminated 7.5 logs of *E. coli* 0157:H7 in beef meat, whereas a 2.5 kGy dose of irradiation was required to eliminated 8 logs of *Salmonella* Enteritidis in chicken meat.

Significance: Results indicated that trisodium phosphate was more effective against aerobic total counts than citric acid. Treatment with trisodium phosphate and citric acid reduced microbial load and pathogens but combination with irradiation eliminated all pathogens.

P1-69 Comparison of Electrostatic Spray, Spray, or Dip Using Lactic Acid, Peroxyacetic Acid, or Beefxide on the Reduction of Rifampicin-resistant *E. coli*

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Introduction: Very small meat processing operations often purchase beef sub-primals for grinding that have not been tested for Shiga toxin-producing *E. coli* (STEC). The use of antimicrobial interventions applied to sub-primals may offer small processors a method to minimize the risk of STEC in ground beef.

Purpose: The purpose was to evaluate the effectiveness of three antimicrobial solutions using three application methods for use in small meat processing facilities. Also, to evaluate the effectiveness of antimicrobial treatments on the mostly fat covered outer surface versus the mostly lean inner surface of the sub-primal.

Methods: The outer fat or inner lean surfaces of beef shoulder clod roasts were inoculated with a five strain cocktail (~5.6 log CFU/cm^2) of Rifampicin resistant *E. coli (E. coli*^{Rif}*).* Three replications of 4.5% lactic acid, 380 ppm peroxyacetic acid, or 2.5% Beefxid were applied at 19-23°C to the sub-primal surface using spray (5 s, 20 psi), dip (15 s), or electrostatic spray (10 s). *E. coli*^{Rif} were enumerated on ACP and *E. coli*/coliform Petrifilm.

Results: All antimicrobial treatments reduced (P<0.0001) *E. coli*^{Rif} counts when compared to the inoculated control (0.39-1.13 log CFU/cm^2 reduction). However, dip and spray applications were more effective at reducing *E. coli*^{Rif} as compared to electrostatic spray (P=0.0008). The reduction of *E. coli*^{Rif} counts were greater on the outer fat versus the inner lean surface of the roast (P=0.0054). Microbial samples of ground beef produced from the clod roasts showed that *E. coli*^{Rif} counts for only spray and dip treatments were different from the control. Ground beef *E. coli*^{Rif} counts were higher from roasts treated on the lean surface versus the fat surface when measured by *E. coli*/coliform Petrifilm (P=0.0001) and ACP Petrifilm (P<0.0001).

Significance: Small meat processors can select an antimicrobial treatment to reduce the STEC on the surface of beef sub-primals and in ground beef.

P1-70 Thermal Inactivation *D* and *z*-Values of *Salmonella* in High-fat Raw Materials for Rendering

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Introduction: Recently, pet food has been recognized as a public health risk due to *Salmonella* presence. Animal by-products can be a source of the pathogen if improper thermal processing or re-contamination of rendered products occurs. There are no current safety standards to limit the amount of contamination in meat processing by-products.

Purpose: To determine the *D*- and *z*-values to inactivate a *Salmonella* cocktail in high-fat raw materials from animal processing by-products subjected to thermal rendering conditions.

Methods: A cocktail of 5 *Salmonella* strains isolated from cattle or associated with human illness were used to prepare an inoculum (8 log CFU/ml). Samples of high-fat beef trim and fat rendering raw material were obtained from local sources, mixed, and inoculated with the *Salmonella* cocktail. The samples were packed in aluminum bags and immersed in an oil bath in the established temperatures range from 60°C to 121°C. Surviving *Salmonella* were enumerated by plating serial dilutions in duplicate onto XLD plates with a thin-layer overlay of TSA for recovery of injured cells and incubated at 37°C for 24 h. The *D*-value of *Salmonella* at each temperature was calculated from the negative inverse slope of the log (CFU/g) vs. time plot. The *z*-value was determined from the negative inverse slope of the log (D) vs. temperature plot.

Results: The thermal death time curves indicate *D*-values for 60 to 121°C ranging from 2.17 to 0.099 min in the high-fat rendering raw material. The *z*-values were 38.61 and 40.82°C, for low and high temperature treatments.

Significance: Thermal lethality data for *Salmonella* reductions in high-fat rendering raw materials will assist renderers to design adequate thermal processes to support critical control points to ensure safety in products used as ingredients in pet food.

P1-71 Thermal Inactivation of *Escherichia coli* O104:H4 in Ground Beef Supplemented with Citral

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Introduction: Use of heat to kill bacteria is the most effective intervention strategy to ensure microbiological safety of thermally processed foods. Pathogens may survive in undercooked products and can result in food poisoning outbreaks.

Purpose: We investigated the heat resistance of a three-strain mixture of *E. coli* O104:H4 in raw ground beef with added citral (0 to 3%, wt/wt).

Methods: Inoculated meat was packaged in sterile bags and were completely immersed in a circulating water bath stabilized at 55, 57.5, 60 or 62.5°C for a predetermined period of time. The surviving cell population was enumerated by surface plating meat samples on tryptic soy agar with added 0.6% yeast extract and 1% pyruvate. Survival equations (log-linear and non-log-linear Weibull models) were fitted to the experimental data.

Results: The Weibull model consistently offered more accurate fit to all survivor curves; root mean square error of the log-linear model was 0.63 versus 0.38 for the Weibull model. Using the linear model, time to 4-log reduction were 165.0, 86.2, 14.7 and 3.5 min at 55, 57.5, 60 and 62.5°C, respectively; the corresponding times using Weibull model ranged from 164.8 at 55°C to 3.2 min at 62.5°C. Supplementing ground beef with 1 to 3% citral rendered the pathogen more sensitive to the lethal effect of heat.

Significance: The results provide food industry with an option to supplement 1 to 3% citral in ground beef and design reduced time and temperature for cooking, that ensures adequate degree of protection against *E. coli* O104:H4 and at the same time, provide energy conservation and quality products.

P1-72 Thermal Inactivation of Avian Virus Surrogates in Aged Chicken Litter

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Introduction: The epizootic viral diseases, linked to highly pathogenic avian virus, have been increasing in poultry production system. The raw or under-treated poultry waste appears to be a reservoir for the spread of the pathogen. In order to establish the sanitization guideline, the uses of bacteriophage as a virus surrogates need to be investigated.

Purpose: The objective of this study was to determine the inactivation of bacteriophages FO, MS2 and ø6 as avian virus surrogates during thermal treatment of chicken litter.

Methods: Aged chicken litter with 30% moisture content was inoculated with *Salmonella* phage FO, *Escherichia coli* phage MS2 and *Pseudomonas* phage ø6, separately. Afterwards, samples containing bacteriophages were distributed inside a glass tube and placed into hotplate at 75°C and 85°C up to 3 h. The subsamples were taken at the selected time intervals, and the titers were determined by 10-fold serial dilution and enumerated according to the double-layer agar method using the host strain for each phage.

Results: The come-up times for heating chicken litter with 30% moisture contents at 75°C and 85°C ranged from 6-9.5 min. The bacteriophage ø6 was undetectable after come-up time by direct enumeration at both temperatures. A non-liner thermal inactivation curve was observed for both FO and MS2. For the liner part of the curve, the *D*-values of bacteriophages FO and MS2 were calculated as11.90 and 5.49 min at 75°C, respectively, and 11.07 and 4.72 min at 85°C, respectively. Clearly, both FO and MS2 are more heat resistant than avian virus based on the published *D*-values.

Significance: Our results demonstrated that using bacteriophages FO and MS2 as surrogates for avian virus can guarantee a large safety margin for thermal processing of chicken litter.

P1-73 Effect of Fat Content and Freezing of Beef Burgers on the Transcriptional Profile of *Escherichia coli* O157:H7 Prior to and after Heating

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Introduction: Thermotolerance of *Escherichia coli* O157:H7 during cooking of beef burgers may be affected by the previous frozen storage conditions and thermal conductance parameters.

Purpose: To assess, at transcriptional level, the effect of fat content and frozen storage duration of beef burgers on the thermotolerance of *E.coli* O157:H7.

Methods: Beef patties of 3 cm thickness were prepared from lean ground beef, with or without the addition of fat (30%), containing 0.5 g of inoculated ground beef with 10^9 CFU/g *E.coli* O157:H7 in the center and stored at -28°C. Thermal process took place on the 1st and 20th day of storage, without thawing, in a preheated (200°C) oven broiler to internal temperature of 50°C (n=2x2). Normalized relative quantification (NRQ) of heat/ cold shock- (*groEL, dnaK, cspA*), stress- (*rpoS*) and virulence- (*stx1, stx2, eae*) related genes was assessed along the i) freezing curve (20°C, 1°C, -1°C, -28°C), ii) heating curve (-28°C, -1°C, 1°C, 50°C) after 1 and 20 days (-28°C) using Real-time PCR (n=2x2).

Results: Freezing followed by thermal processing at 50°C had no significant effect (P>0.05) on *E. coli* O157:H7 population. Cryoprotective role of fat was demonstrated by the delayed transcriptional response of the pathogen regarding *rpoS* and *cspA* along the freezing curve. Particularly, *cspA* was upregulated ($\log_2$ NRQ=2) as soon as -28°C was reached, while in low fat burgers maximum NRQ ($\log_2$ NRQ=3) was observed after 1 day at -28°C. With regards to *rpoS*, upregulation ($\log_2$ NRQ=1.5) was observed after 1 or 20 days at -28°C in low and high fat burgers, respectively. During heating of patties the slight upregulation of *dnaK,* only after short-term storage of low fat burgers, could be indicative of the intensity of heat stress experienced by the pathogen and thus, may partially explain the increased heat tolerance of *E.coli* after prolonged freezing and in the presence of fat.

Significance: These results could contribute to further elucidate the correlation between frozen storage and subsequent thermotolerance of *E.coli* O157:H7.

P1-74 Viability of Shiga Toxin-producing *Escherichia coli* and *Listeria monocytogenes* in Çiğ Köfte, a Traditional Turkish Spicy Meatball, during Refrigerated Storage

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Introduction: Çiğ köfte is a spicy Turkish meatball that is typically prepared with raw beef and consumed as raw. Meat tartare products, such as çiğ köfte, when consumed raw may cause illness.

Purpose: Monitor viability of Shiga toxin-producing *Escherichia coli* (STEC) and *Listeria monocytogenes* (Lm) in çiğ köfte during refrigerated storage.

Methods: Finely-ground beef (93:7% lean:fat) was separately inoculated (ca. 4.0 log CFU/g) with an eight-strain cocktail of STEC or a five-strain cocktail of Lm and then mixed with a traditional blend of bulgur wheat, salt, tomato sauce, diced tomatoes, green peppers, onion powder, fresh garlic, and various spices. Aliquots of buffered vinegar (BV; e(Lm)inateV) or distilled white vinegar (DV; 5% acidity) were added to the inoculated batter at levels of 0.0, 2.5, or 5.0% (vol/wt). The batter was hand-shaped into ca. 15 g balls and stored at 4° or 15°C for up to 3 days.

Results: When çiğ köfte was formulated with or without antimicrobials, pathogen numbers remained relatively unchanged after 3 days at 4°C. In contrast, when formulated without antimicrobials, STEC and Lm levels decreased by ca. 0.3 and 0.7 log CFU/g, respectively, after 3 days at 15°C. When

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formulated with BV, pathogens numbers decreased by ca. 0.2 log CFU/g during storage at 15°C. However, when DV was added to the formulation, reductions of 0.5 and 0.9 log CFU/g in STEC and Lm numbers, respectively, were observed. In contrast, when çiğ köfte was formulated without spices, DV, or BV pathogen levels increased by 0.2 to 0.6 log CFU/g when stored for 3 days at 15°C.

Significance: The çiğ köfte formulated herein did not support growth of STEC or Lm. Our data also highlight the antimicrobial contributions of the spices used to prepare çiğ köfte and the importance of proper handling/storage of the resulting product.

P1-75 Variation in *Campylobacter* Mulilocus Sequence Typing Subtypes Detected on Three Different Plating Media

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Introduction: There are multiple selective plating media available for detection and enumeration of naturally occurring *Campylobacter*. *Campylobacter* produce colonies with differing morphology and characteristics depending on the plating medium used. It is unclear if choice of plating medium can affect the *Campylobacter* subtype recovered from a given sample.

Purpose: The objective of this study was to compare subtypes of *Campylobacter jejuni* and *coli* detected on three discreet selective *Campylobacter* plating media to determine if different media select for different subtypes.

Methods: Fifty ceca and fifty carcasses (n=100) were collected from the evisceration line in a commercial broiler processing plant. *Campylobacter* were cultured and isolated from cecal contents and carcass rinses on Campy-Cefex, Campy Line and RF Campy agars. In cases where a positive was found on all three media, one colony of the most prevalent type on each medium was selected. Isolates were analyzed by full genome sequencing and multilocus sequence typing; sequence types were assigned according to Pubmlst.

Results: A total of 49 samples were positive for *Campylobacter* on all three media. Forty samples had only *C. jejuni;* 2 had only *C. coli* and both were detected in 7 samples. Approximately 71% of samples had the same *Campylobacter* subtype on all three media. Significantly fewer (26%, $P<0.01$) samples had one medium with a different subtype than the other two. When two subtypes were detected, the medium with the odd subtype was Campy Cefex 5 times, Campy Line 3 times and RF Campy 5 times. In only one sample were three different subtypes detected.

Significance: In most cases, all three plating media allowed detection of the same subtype of *Campylobacter* from complex naturally contaminated chicken related samples.

P1-76 Rapid Detection of *Campylobacter jejuni* in Poultry Products Using a Piezoelectric Immunosensor Integrated with Magnetic Immunoseparation

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Introduction: *Campylobacter jejuni* is one of the leading causes of foodborne human gastrointestinal disease worldwide. Poultry and poultry products have been identified as the major transmission routes to humans for this pathogenic bacterium.

Purpose: The objective of this research was to develop a rapid and sensitive method for detection of *C. jejuni* in poultry products based on a quartz crystal microbalance (QCM) immunosensor using magnetic nanoparticles (MNPs) for separation and gold nanoparticles (GNPs) for detection.

Methods: The QCM sensor in a flow cell was prepared by immobilizing the mouse anti-*C. jejuni* monoclonal antibody (mAb1) on the sensor surface to specifically capture *C. jejuni*. Rabbit anti-*C. jejuni* polyclonal antibody (pAb1) was conjugated with MNPs to capture *C. jejuni* in food samples and separate them from food matrices. MNP-pAb1-*C. jejuni* complexes were injected into the flow cell to bind with the mAb1 immobilized on the QCM sensor surface. The goat anti-rabbit IgG antibody (Ab2) conjugated with GNPs were injected into the flow cell to bind with the pAb1 on MNPs. Finally, the resonant frequency changes were measured with the QCM sensor and correlated to the cell number of *C. jejuni*. The specificity of this immunosensor was confirmed with different strains of *Campylobacter* and other pathogens possibly associated with poultry products such as *Salmonella* Typhimurium and *Salmonella* Enteritidis. Broiler carcass wash and ground turkey samples were spiked with *C. jejuni* at different concentrations for use in the tests.

Results: The results showed that the QCM immunosensor could rapidly detect *C. jejuni* in poultry products with a detection limit of 10-30 CFU/ml and the total detection time of < 30 min. The characteristics of *C. jejuni* captured by the antibody immobilized on the surface of QCM sensor were visualized using atomic force microscopy (AFM).

Significance: This highly adaptive and flexible technique could provide the poultry industry a more rapid, sensitive, specific, and effective method for detection of major foodborne pathogens in poultry products.

P1-77 Simultaneous Quantification by Real-time PCR of Viable *Escherichia coli* and *E. coli* O157:H7 in Beef after Heat Treatment

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Introduction: Bacteria can be rapidly quantified by determination of DNA from them using real-time PCR (RT-PCR). However, RT-PCR, when used alone, cannot differentiate DNA from live and dead bacteria. We have developed a SYBR green-based RT-PCR procedure in which cells are treated with sodium deoxycholate (SD) and propidium monoazide (PMA) prior to DNA extraction to prevent the amplification of DNA from dead cells with intact and damaged cell membranes. However, the procedure can only quantify one target organism at a time.

Purpose: To develop a RT-PCR procedure for simultaneous quantification of viable *E. coli* and *E. coli* O157:H7 in beef after heat treatment.

Methods: Optimal concentrations of primers and probes targeting the *tuf* gene and *rfb*E gene in *E. coli* and *E. coli* O157:H7, respectively, and a 79-bp synthetic nucleotide fragment used as an internal amplification control (IAC), were determined by comparing the amplification efficiencies resulting from different concentrations. The ubiquity of *tuf* gene in *E. coli* was tested against a total of 118 strains of *E. coli* from beef (97 non O157 and 21 O157). The optimized triplex *tuf*/*rfb*E/IAC assay in conjunction with SD and PMA was used to determine the surviving *E. coli* and *E. coli* O157:H7 in meat juice heated at 52°C.

Results: All 118 strains of *E. coli* were positive for the *tuf* gene. The triplex assay was able to quantify DNA templates of *E. coli* and *E. coli* O157:H7 ranging from 10^6 to $5x10^1$ with amplification efficiencies between 96.8% and 103.0%. After heating at 52°C, cycle threshold (Ct) values, determined by RT-PCR, increased with decreasing viable cell numbers, determined by plating, at 1.78 and 1.86 Ct/log CFU for *E. coli* and *E. coli* O157:H7, respectively.

Significance: The developed triplex RT-PCR procedure could be used for simultaneous quantification of viable *E. coli* and *E. coli* O157:H7 in beef that has gone through heat treatment.

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P1-78 Using Whole Genome Sequencing and Phylogenetic Methodologies to Cluster *Salmonella* Enteritidis Isolates by Source

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Introduction: This study aims to combine epidemiological and genetic sequence data to improve molecular source tracking. One pathogen, *Salmonella enterica* serotype Enteritidis (SE), has historically been highlighted as frequently coming from shell eggs. However, SE attribution among food commodities is made difficult using conventional methodologies (e.g., PFGE) because of the lack of genetic resolution.

Purpose: I attempted to use whole genome sequencing (WGS) methodologies to differentiate between the closely related SE strains that come from different food commodities to explore genomic regions that are indicative of their sources.

Methods: I used WGS data (e.g., complete genomic sequences and single nucleotide polymorphisms (SNPs)) from SE isolates collected from various food commodities and maintained in GenomeTrakr, which was developed by FDA CFSAN in collaboration with other state and federal labs and houses the genomic sequences of thousands of clinical and environment/food isolates at NCBI. I performed phylogenetic analysis to identify the variability of SE strains among the different food commodities and genomic profiles were then explored to identify genetic loci that differentiated SE sources.

Results: WGS data was collected from over 600 SE isolates in GenomeTrakr according to two parameters: if it (1) came from the United States; (2) had associated metadata indicating geographic location, clinical/environmental status, isolation source (e.g., chicken/egg), and/or other relevant attributes. Preliminary phylogenetic analysis based on SNPs derived from sequence data revealed distinct separation between SE collected from egg and chicken.

Significance: I am currently in the process of determining the exact genetic loci that lead to this separation as well as continuing to develop genomic profile data (e.g., presence or absence of gene/plasmid) to inform sources of clinical cases of salmonellosis. Furthermore, the genomic profiles could be improved as more data becomes available to elucidate the nature of the genetic differences that allow for source specificity.

P1-79 Nitrate Removal in Drinking Groundwater Using N-[(2-hydroxy-3-trimethylammonium)Propyl] Chitosan Chloride (HTCC)

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Introduction: High nitrate nitrogen concentration in ground water can be a threat on human health, especially causing blue baby syndrome in infants. Positively charged chitosan molecules can adsorb negatively charged nitrate nitrogen by ionic binding mechanism; its ion exchange capability appears only in acidic water, but decreases in neutral or slightly acidic water. Therefore it is required to make positively charged chitosan independent of pH. N-[(2-hydroxy-3-trimethylammonium)propyl]chitosan chloride (HTCC) is positively charged chitosan independent of pH at all times.

Purpose: The objective of this study is to investigate the effectiveness of HTCC for removing nitrate from nitrate-spiked aqueous solutions and to examine its adsorption equilibrium and kinetics.

Methods: The kinetics of adsorption at different contact time (0.25 - 360 min) and the equilibrium isotherms at different initial nitrate concentration (1 - 100 mg dm^{-3}), temperature (15, 25 and 35°C), and pH (5.5, 7.0 and 8.5) were investigated. The equilibrium data were fitted using the Langmuir, Freundlich, Sips and Dubinin–Radushkevich adsorption models.

Results: The maximum adsorption capacity was calculated to be 199.6 mg nitrate g^{-1} HTCC for an initial nitrate concentration of 500 mg L^{-1}. Langmuir adsorption isotherms were used to correlate the equilibrium adsorption data. Based on the correlation coefficients, it was concluded that the Langmuir isotherm was more suitable for representing the equilibrium data of nitrate adsorption. In addition, first and pseudo-second order kinetic models were applied to describe the adsorption process. The kinetic parameters for the pseudo-second order kinetics were determined.

Significance: Removal of nitrate nitrogen using HTCC may play a significant role in supplying drinking groundwater at clean level.

P1-80 Arsenic and Lead Concentrations in Shelf-stable Commercial Apple Juices and Fresh Apple Ciders in Michigan

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❖ ***Developing Scientist Competitor***

Introduction: The presence of heavy metals such as arsenic and lead in fruit juices has been raised as a concern because of their toxic nature. Prior surveys of arsenic concentrations in apple juice have not considered the source of the fruit and/or juice concentrate used in the juice manufacture. Therefore, it is unclear if differences exist between juices produced from apple juice concentrate (which, in the US, 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 metals, particularly arsenic (As) and lead (Pb), in samples of shelf-stable apple juice and fresh apple cider obtained retail operations and cider mills throughout Michigan.

Methods: Samples of shelf-stable apple juice (n=17) and fresh apple cider (n=78) were obtained in the fall of 2015. Samples were analyzed for concentrations of arsenic, lead and other metals by inductively coupled plasma mass spectrometry (ICP-MS).

Results: There was a highly significant difference ($P<0.001$) in total arsenic concentrations detected in shelf-stable apple juices and fresh apple ciders. The mean±SEM for As was 2.59±0.36 µg/L in shelf-stable apple juices and 0.37±0.17 µg/L in fresh apple ciders. No samples contained As concentrations in excess of 10 µg/L. Lead concentrations did not differ between shelf-stable apple juices and fresh apple ciders, with mean concentrations of 1.5±0.9 and 1.8±0.4 µg/L in apple juices and ciders, respectively. The maximum Pb concentration detected in any sample was 28 µg/L.

Significance: The As and Pb concentrations detected in this study were consistently less than current advisory levels indicated by FDA for these metals. Fresh apple ciders manufactured using local apples contained significantly lower concentrations of As compared to shelf-stable apple juices, which are largely manufactured using imported apple juice concentrate.

P1-81 Abrin Toxin Stability in Complex Food Matrices

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Introduction: Abrin is an extremely toxic ribosomal inactivating protein commonly found in the tropical plant, *Abrus precatorius*. Although abrin has not been documented in a bioterrorism event, it does not have an antidote, and its lethality, availability, and ease of dissemination make it a

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dangerous foodborne contaminate for bioterrorism activity. A clear understanding of the toxin's stability in foods is necessary in order to determine its overall threat and the potential vulnerability in a variety of foods.

Purpose: This study's objective was to determine the duration of abrin toxin stability in a variety of complex food matrices.

Methods: Hot dogs, liquid eggs, and infant formula were fortified at three levels in four sets at days 7, 5, 3, and 0. Fortifications for low levels were near the limit of detection for each matrix, and high level fortifications were one log higher. Samples were homogenized with GBS and extracted via centrifugation. The aqueous layers were removed and analyzed in triplicate, in accordance with the ELISA manufacturer's instructions.

Results: Calculated absorbance values indicated that at high fortification levels, significant toxin degradation occurs during the initial degradation phase (day 0-3) in hot dogs (62.7%), while significantly lower differences occurred in toxin stability for liquid eggs (20.4%) and infant formula (15.5%). Between days 3 and 5, the differences between absorbance values decreased nearly half from those in the initial phase: hot dogs (33%), liquid eggs (10.6%), and formula (9.1%). Absorbance values stabilized for foods analyzed after day 5.

Significance: The data generated from this study will be used for future proficiency testing purposes and may lend insight into the stability and degradation of abrin toxin, when introduced into a variety of matrices, for food biodefense purposes.

P1-82 ELISA Detection of Gluten in Traditionally Brewed Soy Sauce Samples Obtained during Manufacture

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❖ ***Developing Scientist Competitor***

Introduction: Analytical methods are needed to detect and quantify gluten in ingredients, finished foods and food processing environments. However, detection of gluten in hydrolyzed and fermented foods presents an analytical challenge.

Purpose: This study tracked changes in gluten detection in traditionally brewed soy sauce samples obtained at different stages of production using five commercial gluten ELISA kits.

Methods: Traditionally brewed tamari soy sauces formulated without wheat were produced in a pilot plant and spiked with 0 µg/g, 7.2 µg/g and 72 µg/g gluten at the moromi mash stage of production. Traditionally brewed regular soy sauce formulated with wheat was produced in a pilot plant. Tamari and regular soy sauce samples were collected during various stages of production and analyzed in triplicate using five commercial gluten ELISA kits.

Results: Gluten concentrations in the tamari moromi mash spiked with 72 µg/g gluten were 19 (4% CV) – 31 (3% CV) µg/g, as measured with the Ridascreen sandwich, Ridascreen competitive, AgraQuant G12 sandwich, Aller-Tek sandwich, and Morinaga sandwich ELISA kits. The high sensitivity of the Morinaga ELISA (LOQ, 0.27 µg/g) enabled the lowest detection of gluten in the tamari moromi mash spiked with 7.2 µg/g gluten, while other kits were unable to detect gluten in this sample. Gluten concentrations in tamari sauces after 1-6 months of fermentation were <LOQ values for all ELISA kits. Analyses of regular soy sauce samples indicated the presence of 5,600 (5% CV) – 32,000 (6% CV) µg/g gluten in koji, and 2,100 (0.1% CV) - 18,000 (4% CV) µg/g in the moromi mash. Gluten levels in regular soy sauce after 1-6 months of fermentation were <LOQ values of the ELISA kits.

Significance: This study demonstrates that reliable detection and accurate quantification of gluten, subjected to the fermentation process used in the production of soy sauce, is not possible using available ELISA technology.

P1-83 Assessment of Prolamins from Different Oat Varieties Using R5-Based Sandwich ELISA

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Introduction: Cereal grains contain a protein called gluten consisting of prolamins and glutelins. The prolamin fraction from wheat and related cereals possess sequences that provoke celiac disease (CD). The role of oat-derived prolamins, or avenins, in CD remains controversial. Consequently, many food regulatory entities exclude oats from their gluten classification scheme. In those jurisdictions, enforcement of gluten-free labeling laws requires the use of methods for gluten detection/quantification that selectively report prolamins from wheat, barley, and rye, but not from oats. Currently, most such assay use the R5 monoclonal antibody (mAb). However, it is not known whether R5-based assays or other assays perform uniformly with different oat cultivars.

Purpose: To assess a panel of 19 certified oat cultivars for gluten content using an R5 mAb-based ELISA sandwich assay.

Methods: A panel of 19 oat cultivars were assessed for gluten content using a sandwich ELISA based on the R5 mAb. This antibody binds to epitopes present in triticeae prolamins but absent from avenin from oats. Both native and denatured prolamins were tested. Nested analysis of 3 negative and 3 positive cultivars was performed using Western blot analysis with R5 mAb as well as a non-R5-based LFD assay obtained from Pi Bioscientific. The R5 mAb used in this study was provided under a license agreement from the Spanish National Research Council (CSIS).

Results: Reproducible prolamin detection was seen for only three of the 19 oat cultivars tested (designated 9, 11, and 14) using the R5 ELISA. The R5 positive oat cultivars and a subset of R5 negative oats were additionally tested using Western blotting with R5, as well as with a Skerrit-antibody based ELISA (ELISA SYSTEMS), and LFDs based on novel antibody reagents developed at IEH, all in sandwich format.

Significance: The results obtained from testing select oat cultivars highlight features of existing gluten detection systems in relation to avenin detection which should be considered when performing gluten analysis in oats.

P1-84 Development and Characterization of a Novel Monoclonal Antibody Directed against Gluten

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Introduction: The prolamin fraction from wheat and related triticeae cereals exhibits immunopathogenic potential, and consumption of these grains is associated with coeliac disease. The "gold standard" used by the food industry relies on the use of immunodiagnostic tests based on the R5 monoclonal antibody (mAb). However, gluten detection systems based on this mAb have several shortcomings.

Purpose: In an effort to obtain novel serological reagents with improved specificity profiles, we generated monoclonal antibodies. Herein we describe the immuno-reactive profile of the candidate clones and compare their binding activity against the commercially available R5 mAb.

Methods: A synthetic peptide based on a duplicated and variably "deamidated" version of the canonical R5 binding site (L{Q/E}P{Q/E}{Q/E}PFP{Q/E}{Q/E}L{Q/E}P{Q/E} {Q/E}PFP{Q/E}{Q/E}A was used to immunize Balb/C mice. Tertiary boosts were performed using deamidated gliadin. Colonies were screened based on reactivity towards gliadin (wheat), deamidated gliadin (dg), hordein (barley), secalin (rye), and avenin (oat) using indirect ELISA. IgG+ clones with desirable reactivity profiles were studied by western blot analysis and used to develop sandwich ELISA.

Results: Using a combined vaccine approach, 4 candidate hybridoma clones were generated. Of these, 2D4 demonstrated high, and near equivalent activity against gliadin, hordein, and secalin; with no observed cross-reactivity against avenin (R5(-)), zein, orzenin, and soy. Western blot analysis of 2D4 demonstrated a pattern of reactivity that mirrored that of the R5 mAb. Competitive inhibition analysis suggested that 2D4 and R5 recognize similar epitopes. Sandwich ELISAs based on R5 and 2D4 showed that 2D4 reacted with deamidated gliadin more so than R5, had a more uniform reaction stoichiometry than did R5, and did not react with soy protein.

Significance: Of the clones tested, 2D4 demonstrated the most potential for use in future gluten detection systems, including use in sandwich ELISA for detection of wheat, barley, and rye-derived prolamin residues.

P1-85 Evaluation of a Newly Developed Triple Buffered Peptone Broth for Detection of *Salmonella* in Broiler Feed

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Introduction: Lactose broth (LB) and buffered peptone (BP) are used as pre-enrichment media to recover *Salmonella* from feed. Bacterial utilization of feed carbohydrates results in the production of acidic byproducts causing a drop in the media pH which can injure or kill *Salmonella* and yield false negative results.

Purpose: The objective of this study was to evaluate a new triple buffered peptone (TBP) against LB and BP for the recovery of *Salmonella* from inoculated broiler feed.

Methods: In each of two replications, triplicate 5-g samples of non-medicated broiler feed was individually inoculated with approximately 70 cells/gram of one of four nalidixic acid resistant *Salmonella* serovars (Enteritidis, Heidelberg, Kentucky or Typhimurium, *n*=6 per serogroup). Samples were incubated (24h, 37°C) in 45 mL of broth (LB, BP or TBP). After incubation, the pH of the broth was measured and all broths were streaked on brilliant green sulfa agar containing 200 ppm nalidixic acid, incubated overnight at 37°C and evaluated for the presence of typical *Salmonella* colonies.

Results: The mean pH of TBP pre-enrichment media containing feed (6.6) was significantly ($P<0.01$) higher than BP (5.1) or LB (4.4). *Salmonella* was recovered from all (24+/24) samples pre-enriched in TBP. All samples (24+/24) pre-enriched in BP yielded positive *Salmonella* results. However, significantly fewer ($P<0.01$) samples pre-enriched in LB were found to be positive (2+/24). One positive sample was a *Salmonella* Typhimurium and one was a *Salmonella* Heidelberg.

Significance: Broiler feed pre-enriched in LB or BP suffers a significant decrease in pH during incubation compared to TBP. Pre-enrichment of feed in LB results in lower recover of inoculated *Salmonella* than either BP or TBP. TBP showed promise as a means to maintain near neutral pH in feed samples during pre-enrichment and may allow the accurate detection of *Salmonella* in feed and other similar samples.

P1-86 Optimization of Enrichment Broth for the Detection of *Salmonella* in Spices (Garlic, Onion, Cinnamon, Chili Pepper Powders) and Tea

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Introduction: Recent studies have reported that as much as 7% of imported spices are contaminated with *Salmonella*. Detection of *Salmonella* in contaminated spice samples is often problematic due to the presence of inhibitory compounds in the spices which hinder enrichment of *Salmonella* to detectable levels. There is a great need for robust enrichment methods that allow for sensitive detection of *Salmonella* in spices.

Purpose: To validate enrichment protocols for the growth of *Salmonella* in spices, and its downstream detection using conventional PCR, within 24 h.

Methods: Twenty-five (25) g of the respective spice (garlic powder, onion powder, chili peppers, cinnamon, and green tea) was mixed with double strength Buffered Peptone Water supplemented with L-cysteine (30 mmol/L) and spiked with *Salmonella enterica* Typhimurium to a final concentration of 20 CFU/25 g. Sample to broth ratios varied from 1:9 (garlic powder, chili peppers and tea), 1:50 (onion powder) to 1:100 (cinnamon). After 24 h of growth at 37°C, 5 ml of the mixture was filtered through cheesecloth and 1 ml of the filtrate was used for DNA extraction. The *Hil-A* gene was amplified by conventional PCR as an indicator of pathogen presence. Amplicons were visualized by gel electrophoresis.

Results: After 24 h of incubation, more than 1.0×10^5 CFU/ml of *Salmonella* was recovered for each spice studied. The pH value of each enrichment varied but remained above 5.25. Addition of cysteine to the broth allowed the recovery and growth of *Salmonella* in garlic powder, and had no influence on *Salmonella* growth in other spices, as evidenced by a PCR amplicon in all reactions.

Significance: Detection of *Salmonella* was possible within 24 h following incubation in 2x BPW+cysteine. This work could allow a more rapid, robust, and sensitive detection method for *Salmonella* in spices, based on a single enrichment step followed by RT-PCR.

P1-87 Recovery of *Salmonella* from Steam and Ethylene Oxide-treated Spices Using Supplemented Agar with Overlay

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella enterica* has been recovered from a number of low water activity foods including spices. Spices may be treated with steam and/or ethylene oxide to reduce microbial loads. Therefore, improved recovery of viable *Salmonella* in treated spices is needed to ensure processes are properly validated.

Purpose: The efficacy of agar overlay with or without media supplementation was examined to improve recovery of *Salmonella* on peppercorn and cumin seeds treated with steam and ethylene oxide.

Methods: Peppercorns and cumin seeds were inoculated with *Salmonella*, dried to 0.3 to 0.4 a_w, and treated with steam under vacuum (65.4 to 69.7°C) for 15 s dwell time. Following steam treatment, spices were stomached for 1 min in 0.1% peptone + 0.1% Tween, serially diluted, and plated onto TSA, XLT4 or TSA with added supplements followed by XLT4 overlay. TSA was supplemented with sodium pyruvate (9.09 mmol) + yeast extract (0.6%) [SPY], 3,3'-thiodipropionic acid (5.6 mmol)[TDP], glycerophosphate (10 mmol)[GP], lactate (10 mmol) [LAC], or mannitol (100 mmol) [MAN]. Plated samples were incubated (37°C) for 3 h then molten XLT4 medium was used to overlay. Plates were then incubated at 37°C for 21 h prior to colony enumeration. For peppercorns and cumin seeds subjected to ethylene oxide treatment, TSA was supplemented with SPY, TDP, GP, ATP (8.34 mmol), guanine (64.5 mmol), or magnesium (52.5 mmol). Samples were plated as described above.

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Results: TSA supplemented with SPY or TDP resulted in better recovery of *Salmonella* from steam-treated peppercorns ($P<0.05$). No supplement was associated with improved recovery of *Salmonella* on cumin seeds ($P>0.05$) following steam treatment. For ethylene oxide-treated peppercorns and cumin seeds, no supplement improved recovery of *Salmonella* ($P>0.05$)

Significance: For steam-treated peppercorns, supplementation of TSA with sodium pyruvate + yeast extract or 3,3'-thiodipropionic acid may improve recovery of *Salmonella* when overlay plating methods are used.

P1-88 Development of a Rapid Method to Quantify *Salmonella* Typhimurium Using a Combination of MPN and qPCR with a Shortened Enrichment Time

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Introduction: Since *Salmonella* spp. are one of the more prevalent pathogens causing foodborne disease in humans, thus it is of public health considerable concern to the food industry. Conventional plating methods based on selective and differential media or most-probable-number (MPN) methods to quantify *Salmonella* are labor and time intensive, therefore, it is critical to develop more rapid and accurate detection methods that allow for quantification.

Purpose: In this study, a detection method was developed to quantify *Salmonella* Typhimurium using MPN combined with qPCR and a shortened time enrichment (MPN-qPCR-STE).

Methods: For *Salmonella* Typhimurium enumeration, 10-fold dilutions of cell suspension was transferred into three wells on a microtiter plate (same as three-tube MPN assay) and the plate was subsequently incubated at 37º for 4 h. After nonselective enrichment with nutrient broth (tryptic soy broth), the presence of *Salmonella* Typhimurium in each well was identified using a qPCR and bacterial populations were determined based on calculating MPN. Bacterial cell populations were also determined with various quantification methods including conventional MPN, plating methods, and qPCR alone to compare across independent methods.

Results: The coefficient of determination (r^2) between MPN-qPCR-STE and the conventional MPN exhibited a high level of correlation (0.9752), suggesting that the MPN-qPCR-STE offers a reliable alternative method for *Salmonella* Typhimurium quantification. Although conventional plating and qPCR were limited in their ability to detect low population levels of *Salmonella* Typhimurium (e.g., 0.18 log MPN/ml), these levels could be successfully detected with MPN-qPCR-STE.

Significance: The obvious strengths of the MPN-qPCR-STE are that 1) further confirmation step is not required, 2) the detection limit is as low as conventional MPN, but 3) is more rapid, requiring approximately 8 h to simultaneously complete identification and quantification. This method can be utilized to rapidly quantify *Salmonella* Typhimurium saving both time and labor and may be particularly useful for the food industry and related applications where quantification is important.

P1-89 Early Detection of *Salmonella* spp. Contamination in Raw Beef Meat Samples

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Introduction: *Salmonella* spp. is an important adulterant bacteria found in raw beef meat samples. The cultural methods, based on two successive enrichment steps (MLG 4, ISO 6578), provide a result in 3 and 5 days, for negative and positive samples, respectively. Many alternative qPCR-based methods, certified by AFNOR and/or AOAC, have been developed and typically rely on a short enrichment step (10 ± 2 h) enabling to get a result in 1 to 2 days.

Purpose: In order to reduce time to result to as short as 4 h, we developed a new protocol using the GeneDisc® PCR based technology from Pall Corporation.

Methods: Briefly, 78 fresh raw beef samples were spiked with injured *Salmonella* spp. cells. The spiking dose ranged from 1 CFU/sample to 1 CFU/g and the injury level was comprised between 33 and 79 %. Artificially contaminated samples were enriched in BPW pre-heated at 41.5°C. Enriched samples were collected every hour from 2 to 5 h incubation to perform qPCR analyses. Confirmation of presumptive positive samples was done by sub-culturing in RVS then plating onto XLD agar media or direct plating.

Results: Results highlighted that the sample type, the *Salmonella* strain or its injury level had no impact on the enrichment time. Thus, the enrichment time for the detection of *Salmonella* spp. in raw beef samples only depended on the initial *Salmonella* spp. contamination level. A sample contaminated at 1 CFU/g was called positive by the GeneDisc method after 2 h of enrichment while detection of a sample contaminated by as low as 1 CFU/sample required to be enriched for 5 h.

Significance: Beef meat processors can now sort their incoming meat to a specific process based on an informed decision about contamination. This generates an increased profitability by reducing end-product scrap.

P1-90 Withdrawn

P1-91 Validation of a FDA-developed Multiplex Real-time Quantitative PCR (qPCR) for the Identification of *Salmonella* Enteritidis Using ABI 7500 Fast System

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Introduction: *Salmonella enterica* subspecies *enterica* serovar Enteritidis (*Salmonella* Enteritidis) has become the primary cause of salmonellosis worldwide, accounting for 36% of the 403 outbreaks in 1998 to 2008 in United States. Contaminated eggs and poultry are major sources of human *Salmonella* infections; however, outbreaks have also been associated with beef, turkey, sprouts, nuts, leafy and vine stalk vegetables.

Purpose: To validate a FDA-developed multiplex real-time qPCR using ABI 7500 Fast system for identifying *Salmonella* Enteritidis isolates and detecting *Salmonella* Enteritidis in food and environmental samples.

Methods: Our real-time qPCR assay detects the presence of a 262-bp fragment of the *Salmonella*-specific *invA* gene and a 159-bp fragment of the *Salmonella* Enteritidis-specific SDF gene using custom-designed primers and TaqMan probes, along with a custom-designed internal control. We tested 105 *Salmonella* Enteritidis strains (15 phage types), 130 non-Enteritidis *Salmonella* strains (126 serotypes), and 30 non-*Salmonella* strains. Assays on pine nuts naturally contaminated with *S*. Enteritidis and chicken house drag swabs artificially contaminated with *S*. Enteritidis were performed on 24-h preenriched cultures.

Results: The assay correctly identified all 105 *Salmonella* Enteritidis. Three strains of the 130 non-Enteritidis *Salmonella* tested positive for the SDF genes: one of two *Salmonella* Give strains, one of two *Salmonella* Typhimurium strains, and one of two *Salmonella* Nottingham strains. These anomalous strains have been sequenced for further analysis. All 30 non-*Salmonella* were negative for the SDF genes. Our 24-h qPCR results from pine nut and drag swab samples showed 100% agreement to the results of BAM culture analysis and molecular serotyping assays.

Significance: This validated real-time qPCR will provide FDA an effective tool for specially detecting *Salmonella* Enteritidis in high-throughput food and environmental samples.

P1-92 Isolation and Characterization of New *Salmonella* Enteritidis-specific Bacteriophages as a Bio-recognition Element

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❖ ***Developing Scientist Competitor***

Introduction: *Salmonella* is one of the most common foodborne pathogens and causes severe illnesses including typhoid, enterocolitis and bacteremia. For the prevention and control of salmonellosis, the timely and rapid detection of *Salmonella* in food is required. Bacteriophages are necessary for on-site applicable biosensor method as bio-recognition elements for detection.

Purpose: The purpose of this study was to isolate and characterize new bacteriophage as a bio-recognition element for the development of on-site applicable biosensor.

Methods: Bacteriophage against *Salmonella* Enteritidis (referred as KFS-SE) was isolated and purified from waste water of chicken plants using a plaque assay and CsCl gradient ultracentrifugation. A selectivity study of the KFS-SE was performed with 14 other competitive foodborne pathogens using a dot assay. The morphological characteristics of the KFS-SE were investigated by using TEM. One-step growth curve of KSE-SE was investigated using the plaque assay in order to determine the latent period. Stability of the KSE-SE was examined under various range of pH and temperatures, and at various organic solvent (chloroform, diethylether and ethanol) for employing isolated bacteriophages.

Results: KFS-SE bacteriophages against *Salmonella* Enteritidis was isolated and purified and its final concentration was determined to be 3.57×10^{12} PFU/ml. KFS-SE bacteriophage showed the excellent selectivity against *Salmonella* Enteritidis only. The TEM analysis revealed that the KFS-SE consisted of a icosahedral head (64.3 ± 3.70 nm) and a long contractile tail (239 ± 7.21 nm). The latent time of the KFS-SE was calculated to be 20 min. The optimal ranges of pH and temperature were determined to be pH 3-11 and 4-60ºC, respectively. Any detrimental effect of the KFS-SE on its lytic activity did not observed in various organic solvents, except for ethanol treatment.

Significance: Therefore, the KFS-SE showed sufficient possibility as a new bio-recognition element for the rapid and on-site applicable biosensor method.

P1-93 Validation of 3M Molecular Detection System Compared to the Australian Standard Cultural Method for Detection of *Salmonella* in Water Matrices

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Introduction: The 3M Molecular Detection System utilizes a combination of isothermal DNA amplification and bioluminescence to detect targeted pathogens following an enrichment step. Previously the system has been shown to provide equivalent results to standard methods when testing food and environmental samples.

Purpose: The project aim was to evaluate the 3M Molecular Detection Assay (MDA) *Salmonella* for screening water samples and to compare the results obtained to the Australian standard method.

Methods: Water samples analyzed in this study were "as-received" from clients; some of the samples were artificially inoculated with *Salmonella* to provide positive results. An aliquot of each sample was vacuum filtered and enriched in Buffered Peptone Water for 18-24 h and then tested by two methods: (a) 3M MDA Salmonella as per the manufacturer's instructions; and (b) Australian Standard cultural method via subculturing into MKTTn and RVS, further selective enrichment incubation for 24 h and 48 h and plating of the selective enrichments onto two different selective agars.

Results: Of the 222 samples tested for *Salmonella,* 49 were positive by the 3M MDA and Australian standard method while 173 were negative by both methods. This shows 100% agreement between the two methods under the test conditions used.

Significance: The 3M MDA Salmonella provides a presumptive result for the presence of *Salmonella* in water, within 24 hours, allowing for more timely reporting of results without compromising accuracy.

P1-94 Detection of *Salmonella* in Powdered Gelatin: Comparison of 3M Molecular Detection Assay – *Salmonella* and 3M Molecular Detection Assay 2 – *Salmonella* to the Australian Standard Method (ISO 6579)

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Introduction: The 3M Molecular Detection System utilizes a combination of isothermal DNA amplification and bioluminescence to detect targeted pathogens. A new generation of the assay utilizes proprietary nanotechnology to bind inhibitory substances, allowing for faster assay times and improved workflows. Previously the system has been shown to provide equivalent results to standard methods when testing food and environmental samples, however, no data is available for the performance of the system with gelatin.

Purpose: To evaluate the 3M Molecular Detection Assay – *Salmonella* and the new 3M Molecular Detection Assay 2 - *Salmonella* for screening animal based powdered gelatin and comparing the results to those obtained using the Australian standard method (ISO 6579)

Methods: The 3M Molecular Detection Assay methods required the enrichment of samples in Buffered Peptone Water for 18-24 h then processing and analysis as per the manufacturer's instructions. The Australian standard method requires overnight enrichment in Buffered Peptone Water then subculturing into MKTTn and RVS for selective enrichment. After 24 hours, the selective enrichments are then plated onto XLD agar plus an alternative. The samples analyzed in the study were as provided by the manufacturer, and then a portion was artificially inoculated with 3 different *Salmonella* serovars at low levels to provide positive results.

Results: Of the 45 samples tested for *Salmonella* using each system, 36 were positive by each 3M Molecular Detection Assay and the Australian standard method while 9 were negative in each method. The results showed 100% agreement between the three methods under the test conditions used.

Significance: The 3M Molecular Detection Assay - *Salmonella* and the 3M Molecular Detection Assay 2 - *Salmonella* both provide presumptive positive results for the presence of *Salmonella* in gelatin, within 24 hours, allowing for more timely reporting of results and release of product without compromising accuracy.

Poster

P1-95 Evaluation of 3M Molecular Detection Assay (MDA) and 3M Petrifilm *Salmonella* Express (SALX) System for Detection of *Salmonella* in Naturally Contaminated Poultry and Their Processing Environment

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Undergraduate Student Award Competitor

Introduction: In Malaysia, poultry are slaughtered and retail in wet markets under very poor hygienic conditions. Poultry is also considered to be a major source of *Salmonella* in many countries. High prevalence of *Salmonella* in poultry poses an imminent risk to public health. To prevent the occurrence of this pathogen in poultry, effective and efficient *Salmonella* control programs must be adopted throughout the poultry production chain. Rapid or alternative methods are important, as they reduce the detection time for *Salmonella* considerably in comparison to culture-based methods.

Purpose: This research evaluated the efficacy of 3M Molecular Detection Assay (MDA) and the 3M Petrifilm *Salmonella* Express (SALX) System against culture-based method for the detection of *Salmonella* in naturally contaminated poultry and their environment.

Methods: A total of 145 naturally contaminated samples (whole chickens, chicken cuts, environmental swabs and water samples) were obtained from different wet markets in Penang and Kedah, Malaysia. The samples were analyzed for the presence for *Salmonella* using 3M MDA, 3M SALX System and ISO 6579:2002 conventional culture-based method.

Results: Detection of *Salmonella* by both rapid methods was comparable with the conventional ISO method. Highest number of positive samples were detected with conventional method (133/145), followed by 3M MDA (125/145) and 3M Petrifilm SALX System (123/145). The sensitivity, specificity, negative predictive value, positive predictive value and accuracy were 92.23, 91.67, 55.0, 99.2 and 93.1%, respectively for the 3M MDA and 92.48, 100, 55.0, 100 and 93.10%, respectively for 3M Petrifilm SALX System. As both the 3M MDA and 3M Petrifilm SALX System yielded similar kappa results, it indicates substantial agreement with the culture-based method.

Significance: The data from this study therefore support the use of the 3M Petrifilm SALX System and the 3M MDA as rapid and reliable screening methods for *Salmonella* detection in poultry and its processing environment.

P1-96 Development of a Real-time PCR Assay to Specifically Detect *Salmonella* Typhimurium

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Introduction: In EU, *Salmonella* Typhimurium was recently deemed as an adulterant in poultry meat. While the current Kauffmann-White (K-W) serotyping method requires 4-5 days to assess the presence of the pathogen, a real-time PCR method could allow its specific detection within two days either as a rapid screening method or as a typing step after the detection of *Salmonella* spp. However, due to the close genetic relatedness between *Salmonella* serotypes, finding a unique target allowing distinguishing between them using a straightforward PCR assay is a challenging task.

Purpose: The purpose of this study was to identify and assess the value of a molecular target unique to *Salmonella* Typhimurium in order to detect this serotype specifically.

Methods: Height full *Salmonella* Typhimurium genomes found in the Genbank database were selected as targets and 21 non *Salmonella* Typhimurium genomes were selected as non-target sequences. Whole genome alignment and subtraction of regions of similarity was performed using ssGenfinder algorithm. Resulting sequences were then screened against the *Salmonella* WGS Genbank database and sequences yielding no cross-reactions with other serotypes were selected. Real-time PCR assay were designed and tested on a library of *Salmonella* Typhimurium and non-Typhimurium *Salmonella* serotypes.

Results: Two unique sequences were identified as being characteristic of the targeted *Salmonella* Typhimurium genomes. A simplex PCR assay was designed which showed 100% inclusivity on all *Salmonella* Typhimurium strains tested including monophasic variant and >99% on 254 non-Typhimurium *Salmonella* strains. Only one *Salmonella* Paratyphi B strain cross-reacted but not the Java variant.

Significance: These results suggest that it is possible to identify *Salmonella* Typhiumurium based on a unique sequence thus making real-time PCR assay an interesting alternative method for detecting this serotype. Furthermore, the strategy employed for seeking this unique sequence may be applied to other serotypes of interest in the food industry.

P1-97 Rapid Detection of *Salmonella* spp. in 375-Gram Sample Size of Chocolate Products

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Introduction: Alternative rapid screening methods, especially PCR have been developed to detect earlier *Salmonella* contamination in chocolate products. But chocolate is a challenging food matrix as it has high lipid contents and contains polyphenols which inhibit PCR reactions. Usually, DNA purification is required for such products requiring expensive equipment or fastidious protocols. Dedicated simplified protocols for 375-g test portions of chocolate products (including raw materials) have been developed in order to detect *Salmonella* with the new detection method.

Purpose: This study reports an evaluation of a new detection method specific protocol for the rapid detection of *Salmonella* spp. in 375-g chocolate products.

Methods: Ninety-one artificially contaminated samples of 375 g each were tested with the new method. It consists in a single enrichment in BPW (20 hours at 41.5°C). After incubation step, DNA is extracted using mechanical lysis in a dedicated tube: 20 µl 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 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: A total of 75/91 samples were detected positive and 16/91 were tested negative. All samples were confirmed with the ISO 6579 Reference method and no discordance were observed with the new method. The dedicated protocol presented in this study allows to remove PCR inhibitions for all samples tested.

Significance: The bioMérieux GENE-UP method for 375-g chocolate samples does not require any specific purification of DNA that would lead to fastidious or expensive protocols. The new method presented enables a reliable detection of *Salmonella* spp. in about 21 hours, allowing a rapid decision and cost manufacturing savings.

P1-98 Identification and Subtyping of *Salmonella* Isolates Using Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI–TOF) Mass Spectrometry

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Introduction: Subtyping of isolates of the same genus and species is an important tool in epidemiological investigations. A number of phenotypic and genotypic subtyping methods are available; for example, serotyping is the most widely used initial characterization performed on foodborne pathogens, such as *Salmonella enterica* subsp *enterica*. Unfortunately, serotyping is labor-intensive, costly, time-consuming, requires considerable operator skill and a wealth of reagents. A potential alternative to bacterial serotyping is subtyping using Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI–TOF) Mass Spectrometry. This is a library-based approach, offering a rapid, reproducible method for bacterial identification with a high sensitivity and specificity and at minimal cost.

Purpose: The purpose of this study was to determine the feasibility of using MALDI-TOF to differentiate five known *Salmonella* serovars recovered from experimental microcosms inoculated with known strains of *Salmonella*.

Methods: A reference library for each of the five known *Salmonella* isolates was created using a minimum of 30 spectral readings. A total of 965 unknown *Salmonella* isolates were then identified using MALDI-ToF. The identities of the isolates were confirmed using conventional methods (serotyping/PCR). Sensitivity and specificity data were calculated and McNemar's tests used to determine any statistical difference between identification using MALDI-TOF and conventional methods ($P < 0.05$).

Results: MALDI TOF identified all 965 isolates as being *Salmonella* species. Among them, 938 of these isolates were correctly subtyped using MALDI-ToF (Biotyping score ≥ 2.5). Sensitivity ranged from 82 -100% and specificity for all serotypes was approximately 99%. There was no statistical difference between results obtained using MALDI-TOF and conventional methods.

Significance: This study demonstrates that MALDI-TOF is a viable alternative for the rapid identification and differentiation of *Salmonella* subtypes.

P1-99 Development of a Sensitive Aptamer-based PCR Method with Magnetic Immunoseparation for Detection of *Salmonella* Typhimurium in Ground Turkey

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Developing Scientist Competitor

Introduction: Aptamers are single-stranded oligonucleotide ligands that can bind targets with high affinity and specificity. They have been widely studied in the field of diagnostics as alternatives to antibodies due to their favorable features such as easy labeling, temperature tolerance, and lower cost. PCR methods have been used broadly to detect and identify foodborne pathogens because of its simplicity, rapidity, and specificity.

Purpose: The aim of this research was to develop a sensitive aptamer-based PCR method coupled with magnetic immunoseparation for detection of *Salmonella* Typhimurium in ground turkey.

Methods: First, biotinylated polyclonal anti-*Salmonella* Typhimurium antibody was immobilized on streptavidin-coated magnetic nanobeads to capture *Salmonella* Typhimurium. Second, the selected aptamer B5 was added to bind to the surface of *Salmonella* Typhimurium captured by the magnetic nanobeads. Third, the aptamer B5 was released by heating and amplified by PCR. Finally, PCR products were separated with 2% agarose gel electrophoresis and visualized with a UV trans-illuminator.

Results: DNA aptamer specific for *Salmonella* Typhimurium had been selected using quartz crystal microbalance based SELEX. The developed assay was able to detect 10^2 CFU/ml of *Salmonella* Typhimurium in pure culture and 10^3 CFU/ml of *Salmonella* Typhimurium in ground turkey.

Significance: This study demonstrated the feasibility and application of an aptamer PCR method coupled with magnetic immunoseparation for sensitive detection of *Salmonella* Typhimurium in ground turkey.

P1-100 Development and Validation of an Innovative Detection Method for *Salmonella* from Cloves

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Introduction: Detection of *Salmonella* in some spices, such as, cloves remains a challenge due to their inherent antimicrobial properties.

Purpose: The purpose of this study is to develop an effective detection method for *Salmonella* from spices using cloves as a model.

Methods: Two clove varieties, Ceylon and Madagascar, were used in the study. Cloves were inoculated with *Salmonella* ser. Montevideo, *Salmonella* ser. Typhimurium, or *Salmonella* ser. Weltevreden at 1, 3 or 6 log CFU/g. Two sample sizes, 10 and 25 g, were compared. Trypticase soy broth (TSB) was used as preenrichment broth at a broth: clove ratio of 9:1. After adding TSB to cloves for preenrichment, three different preenrichment treatments were evaluated: cloves were left in the preenrichment TSB broth during preenrichment (PreE1); and the mixture was shaken vigorously for 30 (PreE2) or 60 sec (PreE3) and the decants were transferred to a new bag for preenrichment. The rest of the procedures were carried out according to the FDA BAM. Six trials with 4 replicates each were conducted.

Results: All samples from PreE2 and PreE3, at inoculation levels 6 log CFU/25g or 3 log CFU/25g, were positive for *Salmonella*. However, PreE1 only produced 38 positive from a total of 48 samples at inoculation level 6 log CFU/25g. It had 12 positive results among 48 samples at inoculation level 3 log CFU/25g. A similar trend was observed at inoculation level 1 log CFU/25g. Therefore, PreE3 with 25 g cloves/sample was validated by comparing with FDA BAM in 6 trials, with each trial consisting of 20 samples. The results showed the PreE3 detected *Salmonella* from 88 of 120 inoculated samples compared with 31 positive of 120 by BAM.

Significance: In conclusion, the newly designed method PreE3 was substantially superior to the current FDA BAM for the detection of *Salmonella* from cloves.

P1-101 Evaluation of the GENE-UP Listeria (LIS) Assay for the Detection of *Listeria* spp. in Food and Environmental Surfaces

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Introduction: The GENE-UP is a novel real-time PCR system for pathogen detection. This new PCR platform is highlighted by an exceptionally easy to use workflow.

Purpose: The goal of this study was to investigate this alternate *Listeria* method for the detection of *Listeria* spp. in 375-gram food samples and environmental sponges.

Methods: Seven 375-gram samples of hot dogs, deli turkey, RTE beef patties and brie cheese were inoculated with *L. monocytogenes* at a level of approximately 21 CFU/test portion. A single 375-gram sample of each product was tested unspiked. All test portions were enriched in LPT broth (1,125 ml) and incubated for 26 h at 35±1°C. For the environmental samples, stainless steel, ceramic and rubber were inoculated with *Listeria* spp. in a "4 × 4" area at around 10^5 cells/test square. Seven inoculated and one unspiked area were tested for each surface. After inoculation the surfaces were allowed to dry 20 ± 4 h at room temperature before testing. A sponge was then used to sample each surface and 100 ml of LPT broth was added to the individual sponges and incubated for 18 h at 35±1°C. Enrichments for both the food and environmental samples were analyzed by the *Listeria*PCR method. All enrichments were confirmed using the USDA MLG reference method, regardless of initial screen result.

Results: The $dPOD_{CP}$ as the difference between the alternate method presumptive result POD (POD_{AP}) and the alternate method confirmed result POD (POD_{AC}) values were calculated for each product and environmental surface. The 95% confidence interval of the $dPOD_{CP}$ was also determined. For all products and surfaces, the calculated 95% confidence interval contained zero indicating that there was no significant difference between the alternate method and the USDA MLG reference method plating for this study.

Significance: These data demonstrate that the evaluated method is a suitable for detecting *Listeria* spp. in 375-gram food samples and environmental sponges. The method provides significant savings in terms of time and improved convenience when compared to reference methods and other commercially available PCR platforms.

P1-102 Rapid Detection of *Salmonella enterica* from Raw and Roasted Pistachios and Almonds through Loop-Mediated Isothermal Amplification (LAMP) and Bioluminescence

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Introduction: In the last decade, tree nuts have been associated with a number of *Salmonella* outbreaks. In the first half of 2015 in the USA, fifteen recalls, triggered by routine examination, took place due to the presence of *Salmonella*. Detection of foodborne pathogens in this particular commodity is challenging as microorganisms can potentially be stressed due to the low availability of both water and nutrients. Providing methods and technologies that can offer high sensitivity and specificity to recover and rapidly detect pathogenic microorganisms from these matrices is essential.

Purpose: To assess the performance of a rapid method based on LAMP-bioluminescence to detect *Salmonella enterica* from raw and roasted almonds and pistachios compared to FDA/BAM as reference method.

Methods: Portions of 25 g of in-shell raw, kernel meat and roasted pistachios and almonds were inoculated with 1-5 CFU of lyophilized *Salmonella* (*n*=9/nut/method/microorganism; N=108). Non-inoculated controls were also included (*n*=3/nut/method/microorganism; N=36). Inoculated samples and controls were enriched and processed for detection of target organisms following FDA/BAM procedures or were enriched with 225 ml of buffered peptone water ISO and incubated at 37°C/18 h followed by detection of target microorganisms with the LAMP-bioluminescence rapid method. Probability of detection (POD) was calculated to compare the two methods.

Results: The rapid method based on LAMP-bioluminescence was able to detect *Salmonella* from pistachios and almonds after enrichment providing next day results. No significant difference (P<0.05) was determined between the POD of the FDA/BAM and the LAMP-bioluminescence methods.

Significance: *Salmonella enterica* detection through LAMP-bioluminescence, a rapid technology that allows sensitive detection of foodborne pathogens from difficult matrices like nuts, offers this industry a tool to support food safety programs related to pathogen testing.

P1-103 Evaluation of a New Method for the Rapid Detection of *Salmonella* in Large Size Cocoa Samples

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Introduction: *Salmonella* detection is of great concern in the chocolate industry, especially at the level of the raw materials like cocoa mass or cocoa powders. The chocolate manufacturers need rapid methods for testing. The ISO 6579 reference method requires 3 days and main not be appropriated for large size samples. Polyphenolic compounds in cocoa may inhibit the PCR assays, thus preventing the use of this technology that could greatly reduce the time-to-result.

Purpose: The purpose of this study was to evaluate a new method for the detection of *Salmonella* in cocoa product based on a single enrichment in skimmed milk followed by a DNA extraction with a purification solution formulated to neutralize the effect of the inhibitory components and a subsequent PCR assay with the iQ-Check® *Salmonella* kit.

Methods: *Salmonella* stressed strains (heating, 56°C and dehydration, freeze-drying) were used to spike 250 g or 375 g of cocoa containing samples. The spiking levels were below 10 CFU/sample. Spiked samples and control samples were then analyzed in duplicate by the alternative and the standard method including a pre-enrichment in BPW supplemented with dry milk and brilliant green, enrichment in MKTTn and RVS broths and streaking on agar plates.

Results: All the non-spiked samples were negative. A total of 40 spiked samples (high cocoa and low cocoa contents) were tested. All the samples were detected with both, the reference and the alternative methods. The samples treated with the purification solution gave positive results while the non-treated samples showed inhibition of the PCR assays.

Significance: These data show that the new method can be used to detect *Salmonella* in large size samples of cocoa containing product. The enrichment step can be made in skimmed milk which reduces the cost of the enrichment compared to the reference method. The purification solution allows the use of the PCR based iQ-Check® *Salmonella* kit, thus improving also the time-to-result.

P1-104 Simultaneous Enrichment of *Salmonella* spp., *E. coli* O157:H7 and *Listeria monocytogenes* in Leafy Greens

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Introduction: The rapid and reliable detection of *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* is important for prevention of foodborne illnesses. Several food matrices, including produce, are high-risk for contamination with multiple pathogens. As per Bacteriological Analytical Manual (BAM), depending on the food matrix and target pathogen, different pre-enrichment media are used. Using multiple media is labor intensive and a roadblock for multi-pathogen detection screening methods in foods. To facilitate a multi-pathogen detection platform, a universal non-selective pre-enrichment medium is necessary.

Purpose: The objective of this study is to identify the best medium for simultaneous enrichment of *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* in leafy greens for a multiplex qPCR-based detection.

Methods: *Salmonella* Typhimurium, *E. coli* O157:H7, *Listeria monocytogenes* were co-inoculated into 25 g of baby spinach and 225 ml of pre-enrichment broth. Candidate media included BAM media such as Universal Pre-Enrichment Broth (UPB) and Tryptone Soy Broth (TSB), published research broths including, SEL, SSL, a FDA Research Broth (BMW) and modifications of these broths. After 24 h incubation at 37°C, DNA was extracted for multiplex qPCR analysis.

Results: Results show that the target pathogens were simultaneously enriched in spinach using all candidate broths. Ct values were significantly higher (P<0.05) for the enrichment of *Listeria monocytogenes* as compared to *Salmonella* Typhimurium and *E. coli* O157:H7 in all broths with the exception of SEL and mBPW+glucose, whereby lower Ct values for *Listeria monocytogenes* indicate greater enrichment. For example, Ct values for *Salmonella* Typhimurium, *E. coli* O157:H7 and *Listeria monocytogenes* were 26.98±0.58, 26.98±0.58, and 34.67±4.21, respectively, in SSL, as compared to 24.92±0.95, 24.92±0.50 and 29.89±0.65, respectively, in mBPW+glucose.

Significance: The results will improve current regulatory microbiological methods encompassing the recovery, enrichment, and detection of multiple microbial pathogens from foods to provide a less labor intensive means to sample preparation and pathogen screening.

P1-105 Reveal 2.0 for Group D1 *Salmonella* Test for Raw Shell Eggs, Poultry Feed and Chicken Carcass Rinse

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Introduction: Reveal 2.0 for Group D1 *Salmonella* is a lateral flow test device for detection of *Salmonella* Enteritidis (SE). The antibodies used in this device are broadly inclusive for SE and other serovars of the D1 somatic group. In 2014, this test was approved by the National Poultry Improvement Plan for use in testing poultry-house environmental samples.

Purpose: In this study, the Reveal test was evaluated for rapid detection of SE in raw shell eggs, poultry feed and chicken carcass rinse.

Methods: A low level of SE was inoculated into pooled raw shell eggs and a paired analysis was conducted utilizing a common primary enrichment method prescribed in the FDA/BAM procedure. Chicken carcass rinse and poultry feed samples spiked with SE were tested with the Reveal test and the USDA-FSIS/MLG or FDA/BAM reference methods, respectively. The novel secondary enrichment methodology incorporates modified semisolid Rappaport-Vassiliadis broth (MSRV) and the direct application of the Reveal device. For each matrix, 20 replicate test portions were inoculated to produce fractional positive levels, along with 5 high level and 5 uninoculated samples. Statistical analysis was conducted on the Reveal test and reference method data using probability of detection (POD) models.

Results: Method comparison data for fractional positive samples, at a 95% confidence interval, produced a dPOD of 0 (-0.14, +0.14) for raw shell egg samples, a dPOD of 0 (-0.26, +0.26) for chicken rinse samples and a dPOD of 0.3 (0, 0.54) for poultry feed samples. There was no statistically significant difference between performance of Reveal and reference methods. The Reveal test demonstrated inclusivity of 98% for target strains (49/50) and exclusivity of 100% for non-target strains (40/40) tested.

Significance: The study results demonstrate the utility of Reveal 2.0 for Group D1 *Salmonella*, as a rapid test for detection of SE in raw shell eggs, poultry feed and chicken carcass rinse.

P1-106 Detection of *E. coli* O157:H7, Non-O157 STEC and *Salmonella* from a Single 25-g or 375-g Enrichment of Spinach Using the DuPont BAX System

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Introduction: Enteric pathogens such as *Escherichia coli* O157:H7 and *Salmonella* are the leading cause of fresh produce associated diseases. Due to the short shelf life of produce, PCR testing is often used to detect these pathogens to achieve rapid, highly sensitive results. However, plant substances such as polysaccharides and polyphenols present a challenge to these detection systems.

Purpose: The purpose of this study was to determine the fastest time-to-result to detect *E. coli* O157:H7, non-O157 STEC, and *Salmonella* in spinach from a single 25-g and 375-g sample enrichment.

Methods: Spinach portions of 25 g and 375 g (*n*=20 each) were fractionally inoculated with *E. coli* O157:H7, *E. coli* O26 and *Salmonella*, held at 4°C for 72 hours, and homogenized with 100 ml or 1,500 ml, respectively, of pre-warmed (42°C) BAX System MP media. Samples were incubated at 42°C for 8-22 hours and tested directly from the primary enrichment and after a BHI regrowth using real-time PCR. Two additional sets of samples were enriched according to the FDA-BAM reference methods for Diarrheagenic *E. coli* (*n*=20) and *Salmonella* (*n*=20) and tested using real-time PCR to provide a rapid presumptive results in addition to culture.

Results: For 25-g samples, 90 real-time PCR tests were performed with 100% agreement to culture after 10 hours of enrichment with a regrowth. For 375-g samples, 90 real-time PCR tests were performed with >99% agreement to culture after 12 hours of enrichment with a regrowth; statistical analysis demonstrated the difference between methods to be insignificant.

Significance: The results of this study demonstrate that the BAX System can detect all three organisms simultaneously from a single 25-g or 375-g enrichment of spinach in BAX System MP media.

P1-107 Real-time PCR Detection of *Salmonella* Species in Highly Inoculated 325-g Samples of Ground Turkey with a Reduced Enrichment Volume and Shortened Time-to-Result

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Introduction: The natural occurrence of *Salmonella* in poultry and the global increase in ground turkey consumption poses a significant impact on consumer contamination risk. As a result, the poultry industry is under higher scrutiny to reduce contamination in ground product. Current testing programs for raw poultry are cost prohibitive largely due to the sample size (325 g); therefore, establishments are looking to reduce costs by decreasing the 1,625 ml enrichment volume and shortening the time-to-result.

Purpose: The purpose of this study was to assess the ability of a real-time PCR assay to detect *Salmonella* using a reduced enrichment volume for highly inoculated 325-g samples of ground turkey for a shortened time-to-result.

Methods: Six different *Salmonella* serovars prevalent in poultry were selected to highly inoculate 325g samples of 85% (n=30) and 93% (*n*=30) lean ground turkey at a concentration of 10 CFU/g. After holding spiked product for 8-10 hours at 4°C, samples were homogenized with 975 ml of pre-

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warmed (46°C) BPW and incubated at 42°C for 4-10 hours. All samples were analyzed with the DuPont BAX System Real-Time PCR Assay for *Salmonella* and confirmed according to the USDA-FSIS reference culture method.

Results: For both 85% and 93% lean ground turkey samples, real-time PCR detected all positive results (*n*=60) after 6 hours of enrichment for all *Salmonella* serovars consistent with the reference culture method.

Significance: The results of this study provide establishments with a cost savings benefit by using a reduced enrichment method compared to the USDA-FSIS enrichment method of 1,625 ml to quickly determine status of contamination.

P1-108 Multiplex Real-time PCR Assay for Reliable Detection of *Salmonella*

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Introduction: Due to a large number of serotypes and frequent mutations of *Salmonella* virulence genes, finding a mutual gene to detect all *Salmonella* serotypes in one assay is impossible. In addition, some non-*Salmonella* strains, such as *Citrobacter amalonaticus*, will yield false-positive results with single gene detections.

Purpose: The aim of this study was to design a three-gene multiplex high resolution melt-curve (HRM) PCR to detect the majority of *Salmonella* serotypes while avoiding false-positive and false-negative results.

Methods: The *inv*A, *stn* and *fim*A genes were selected due to their widespread presence in all *Salmonella* strains. Primers were designed and tested for each target gene. A melt curve with an internal amplification control was standardized in the assay. This method was tested on 40 *Salmonella* strains and applied in various food samples.

Results: Three specific and separated peaks were formed for most *Salmonella* serotypes in this PCR, which indicated that all three products were formed. Several *Salmonella* strains showed the absence of one peak, but gave positive results for the other two target genes. After a 6-h enrichment, target genes could be detected in 25 g of food samples inoculated with 10^3 CFU of *Salmonella*.

Significance: This assay uses a HRM multiplex PCR rather than a singleplex PCR to detect *Salmonella* serotypes, which reduces the chance of getting false-negative results from *Salmonella* serotypes missing one of the target genes, and avoids false-positive results from non-*Salmonella* strains that possess one of the three genes, making it a highly reliable and accurate method to detect *Salmonella* in foods.

P1-109 Evaluation of the IQ-Check Kits for Detection of Shiga Toxin-producing *E. coli* and *Salmonella* in Ground Beef and Comparison to the USDA Microbiology Laboratory Guidebook Methods

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Introduction: Shiga toxin-producing *E. coli* (STEC) O157:H7 and non-O157 STEC serogroups O26, O45, O103, O111, O121, and O145 are important foodborne pathogens declared as adulterants by the USDA Food Safety and Food Inspection Service (FSIS). Bio-Rad developed real-time PCR kits (iQ-Check) to screen for STEC and *Salmonella*, and the FSIS Microbiology Laboratory Guidebook (MLG) method utilizes the BAX System kits (DuPont). Both screening tests target Shiga toxin (*stx*1 and *stx*2), intimin (*eae*) and O-group-specific genes. The Bio-Rad and MLG methods utilized SEB medium and mTSB, respectively, for enrichment of both STEC and *Salmonella* simultaneously.

Purpose: To compare the MLG and Bio-Rad methods for detection of STEC and both STEC and *Salmonella* simultaneously in ground beef after a short enrichment period, followed by isolation of colonies from selective agars and confirmation.

Methods: Beef samples were artificially contaminated with the STEC strains (4-19 CFU), and STEC (4-16 CFU) together with *Salmonella* Typhimurium (10-15 CFU), kept at 4°C for 72 h, enriched for 12 and 18 h, and then tested with iQ-Check kits and with BAX System kits as described in the MLG. For *Salmonella* isolation, 0.1 ml of enrichments that had been co-inoculated was transferred to RVS broth, incubated for 24 h at 42°C, and then plated onto XLT-4 agar. STEC isolation was carried out using the MLG protocol. Presumptive colonies were confirmed using the real-time PCR kits.

Results: All of the samples were positive by PCR after 12 and 18 h of enrichment, and no notable differences were found between the two methods. Confirmed STEC and *Salmonella* colonies were obtained from every enrichment.

Significance: This study demonstrated that the iQ-Check kit results compared well with the MLG methods, and detection of both STEC and *Salmonella* in beef using the same enrichment medium is possible after a relatively short enrichment period.

P1-110 Performance of a New PCR-based Molecular System for the Detection of *Salmonella* and *E. coli* O157:H7 in a Variety of Food and Environmental Samples

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Introduction: GENE-UP is a new PCR-based molecular system for as early as same day detection of *Salmonella* and *E. coli* O157:H7 in food and environmental samples using a simplified workflow.

Purpose: To assess the performance of alternate method against the reference methods for detection of *Salmonella* and *E. coli*O157: H7 in food and environmental samples.

Methods: The *Salmonella* method evaluation (alternate vs. USDA-FSIS-MLG-4.08) was performed on raw ground beef (25 g and 375 g), raw chicken breast, raw fish, creamy peanut butter, vanilla ice cream, dry pet food (all 25-g samples) and stainless steel (sponges). Alternate method for *E. coli* O157:H7 was compared to the USDA-FSIS- MLG 5.08 for ground beef (25 g and 375 g) and FDA-BAM for spinach (200 g). All results were compared using the probability of detection (POD) following AOAC guidelines.

Results: For samples with low contamination levels (*n*=20/ matrix), the difference in POD for confirmed *Salmonella* alternate and reference methods ($dPOD_C$ values along with 95% confidence interval in parenthesis) were -0.15 (-0.41,0.14) for 25-g beef samples and -0.15 (-0.41,0.15) for 375-g beef samples; 0.15 (-0.15, 0.41) for chicken breast; 0.05 (-0.24, 0.33) for fish; 0.15 (-0.13, 0.40) for peanut butter; 0.10 (-0.19, 0.37) for ice cream; 0.15 (-0.15, 0.41) for pet food; and 0.05 (-0.23, 0.33) for steel surface samples. For *E. coli* O157:H7, $dPOD_C$ values were 0.15 (-0.11, 0.39) for 25 g beef; -0.10 (-0.37, 0.20) for 375-g beef; and 0.15 (-0.13, 0.40) for 200-g spinach samples. The confidence interval of all above $dPOD_C$ values as well as for all un-inoculated and high contamination level samples (*n*=5 each/ matrix) contained zero, showing no statistical difference between the compared methods.

Significance: No performance difference was found between the alternate and reference methods. The alternate method employs a simplified workflow and offers a significant time saving in comparison to the reference methods.

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P1-111 Whole Genome Assembly (WGA) of *Salmonella* from Shotgun Metagenomic Samples Directly out of Spice-enriched Mixed Cultures (EMCs) Using Current WGS Analysis Tools

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Introduction: Current molecular epidemiological practices use WGS assemblies from pure cultures to analyze and compare foodborne outbreaks. A metagenomic approach from enriched mixed cultures (EMCs) of complex food matrices like spices may result in earlier identification of pathogens.

Purpose: The purpose of this study was to determine the possibility of achieving sufficient depth of coverage and substantial recovery of WG to assemble and identify foodborne pathogens like *Salmonella* from spice metagenomic datasets using WGS analysis tools.

Methods: White pepper (WP) samples implicated in a 2009 *Salmonella* outbreak were processed following a modified BAM protocol. Qiacube extracted DNA was collected from samples throughout enrichment. Shotgun metagenomic sequencing was performed on the MiSeq using the Nextera kit. *Salmonella*-specific reads were collected by mapping and assembled using CLC Workbench. Assemblies were also generated from WGS of *Salmonella* isolated from WP in our laboratory and from outbreak-associated NCBI archived reads. Core gene MLA and RAST were used to compare the metagenomic assembled genome (M-A) with those of the isolated colony (IC-A) and NCBI entries (NCBI-A). Additionally, Metaphlan2 analysis was performed. A similar approach was applied to spice samples spiked with *Salmonella*.

Results: Ability to assemble WG from metagenomic datasets by applying WGS analysis tools was demonstrated using a naturally contaminated spice sample. *Salmonella* was detected by microbiology and bioinformatics at various stages of enrichment. Metaphlan2 results provided relative abundance of *Salmonella* in the samples. The M-A was virtually identical to IC-A and to the NCBI-A. WG recovery from spiked samples was proportional to the abundance of *Salmonella* detected in the sample by Metaphlan2. Enrichment conditions and initial *Salmonella* load appear to affect WG recovery from metagenomics data sets using this approach.

Significance: Good quality WG assemblies directly from EMCs may provide rapid, high resolution molecular epidemiological data for detection and source tracking of pathogens in food.

P1-112 Real-world Assessment of Process Control Utilizing a Poultry Rinse Limits Testing Application with Paired Qualitative Method Comparison for Detection of *Salmonella enterica* in the Poultry Production Environment

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Introduction: Qualitative *Salmonella* results yield little insight to load and sample preparation may impact accuracy. Alternative methodologies may be useful for monitoring process control and intervention efficacy.

Purpose: This study was conducted to assess a poultry rinse limits test application in real-world production while comparing qualitative detection methods.

Methods: Poultry rinse samples (N=240) were collected over 5 weeks from two plants in the eastern United States. Within each week, sampling was conducted over 4 days, 2 shifts and 3 processing locations including re-hang, pre-chill and parts. One carcass or 4 lbs of parts were rinsed in 400 ml of BPW and shipped at 4°C overnight to a 3rd party laboratory. Rinses were evaluated via 4 diagnostic pathways including the Limits Test (3.5 h enrichment), Undiluted Rinse Qualitative Test (20-24 h), and the USDA MLG 4.08 with paired analyses (20-24 h). The limits test was optimized to detect Salmonella loads of ~ 1.30 CFU/ml of rinsate while minimizing detection of loads below. Method results were evaluated for prevalence across sampling variables and McNemar Chi-square for method comparison.

Results: For 213 complete samples, qualitative prevalence by the MLG 4.08 method across re-hang, pre-chill and parts ranged from 35.29-86.84%, 30.30-80.56% and 14.71-26.32% for plants A and B, respectively. In contrast, direct rinse enrichment qualitative prevalence ranged from 55.88-94.74%, 39.39-91.67% and 23.53-44.74%, respectively. Prevalence of samples above the application limit of ~1.30 CFU/ml ranged from 26.47-57.89%, 15.15-55.56% and 5.88-7.89%, respectively across locations for Plant A and B. *Salmonella* load as observed through limits positive samples trended with prevalence. Finally, no significant differences were observed between the Roka and BAX qualitative tests following MLG 4.08, however, a significant difference was observed between direct rinse enrichment and MLG 4.08.

Significance: Limits testing may provide an alternative means of monitoring process control and intervention efficacy. Qualitative testing may be impacted by sample preparation.

P1-113 Independent Validation for the Detection of Salmonella enterica in Dry Pet Kibble Utilizing the Atlas *Salmonella* SEN Detection Assay

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Introduction: Increasing food safety emphasis for dry pet kibble necessitates rapid and reliable methods for *Salmonella* detection in the challenging finished product matrix.

Purpose: This study was conducted to validate the Atlas *Salmonella* SEN Detection Assay for the analysis of pet food kibble samples prepared at 1:6 and 1:10 dilution ratios with flexibility in application testing parameters.

Methods: For two studies, 375-g samples were prepared to achieve 5 high replicates (6-10 CFU/375 g), 20 low replicates (0.5-2 CFU/375 g) and 5 un-inoculated controls. Samples were bulk, dry inoculated with a lyophilized pellet of *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028). Samples were enriched with pre-warmed 42°C BPW at a 1:6 or 1:10 dilution for studies 1 and 2, respectively, and enriched for 24 hours at 42° ± 2°C with sampling of 40 and 400 µl into Roka Transfer Tubes at 18, 20 and 24 hours. RTTs were then analyzed by the Roka *Salmonella* SEN Detection Assay on the Atlas instrument. Samples were cultured at 24 hours by direct streak to CHROMagar *Salmonella* and FDA-BAM Ch. 5. **Results** were tabulated and assessed by paired probability of detection.

Results: For study 1, the Atlas SEN Assay detected 12 and 5 presumptive positives in the low and high level replicates, respectively. All results correlated 100% across 18, 20 and 24 hour sampling time points and for 40 and 400 µl transfer volumes. In study 2, the assay detected 13 and 5 presumptive positives, respectively, and results, similarly, correlated 100% across all study parameters. All un-inoculated samples were negative by Atlas and culture. No significant differences were observed by paired POD. CHROMagar *Salmonella* performed equivalently to FDA BAM Ch. 5.

Significance: These data demonstrate flexibility of the Atlas *Salmonella* SEN Detection Assay and Atlas instrument for the rapid and accurate detection of *Salmonella* in dry kibble.

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P1-114 Use of 3M Molecular Detection Assay for the Recovery of *Salmonella* and *Listeria* Species from the Surface of Avocados

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Introduction: *Salmonella* and *Listeria* species are ubiquitous microorganisms. The presence of these pathogens in fruits increases the risk of illness. Outbreaks of *Salmonella* and *Listeria monocytogenes* have in the past been linked to produce. Fast and accurate results on pathogen determination are key in food safety management. Use of technologies such the 3M Molecular Detection Assay (3M MDA) combines isothermal DNA amplification and bioluminescence detection providing real-time detection and next-day results.

Purpose: The purpose of this study was to evaluate the performance of 3M MDA for the recovery of *Listeria* species and *Salmonella* from avocado's surface.

Methods: *Salmonella* Enteritidis (ATCC 13076) and *Listeria monocytogenes* (ATCC 19115) were used to artificially contaminate avocados (0.4-4.0 CFU/avocado) by dipping. Thirty avocados were contaminated and five were used as a negative control for each microorganism. Each avocado was placed in enrichment broth (3M Buffered Peptone Water-ISO for *Salmonella* and 3M Modified *Listeria* Recovery Broth and Supplement for *Listeria*) at a 1:1.5 w:v ratio and incubated during 24 h at 35°C. All the samples were culture confirmed.

Results: A total of 35 avocados were analyzed for each test. Sensitivity was 96.7% for *Listeria* and 100% for Salmonella assays. Statistically significant differences were not detected (P>0.05) when these rapid tests were compared with the respective traditional methods for the recovery of *Salmonella* or *Listeria* species. All the avocados used as control were negative.

Significance: Use of MDA assays for the detection of *Salmonella* or *Listeria* species on avocado surfaces provide fast and accurate results that are equivalent to the traditional method.

P1-115 An Independent Evaluation of Alternative Rapid Methods for the Detection of *Salmonella* in Select Hydrocolloids (Gums)

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Introduction: Low moisture foods are naturally low in moisture content or have been produced through a drying or dehydration process. Their low water activity (a_w < 0.85) has led to the perception that they are low risk for contamination from a microbiological perspective. However, in recent years, a number of foodborne illnesses resulting from ingestion of *Salmonella* has been linked to these commodities. There is a need for industry to ensure that pathogen detection methods are fit for purpose.

Purpose: To conduct an initial feasibility evaluation of the performance of AOAC OMA 2013.03 and 2003.09 for 9 gums using enrichment media modified with an enzyme or various enrichment media-to-sample-volume ratios. A larger method validation study was conducted on one emulsifier upon completion of the feasibility study.

Methods: Feasibility Study: For each matrix, 10 replicates at a high contamination level (5-10 CFU/test portion) were evaluated by the alternative RT-PCR methods. Method Validation: Thirty sample replicates (20 low level, 5 high level, 5 uninoculated control) were evaluated in a paired study by the alternative method and a modification of the FDA/BAM Chapter 5 reference method.

Results: No issues were observed for the 9 matrices during the feasibility evaluation. GHB, a combination of two gums (LBG and Guar) evaluated in the feasibility study was selected for the full method validation. Using the POD statistical model, no statistical significant difference was observed between the alternative and reference methods.

Significance: The data generated in this evaluation indicates that the rapid alternative methods with modifications to the enrichment media are a suitable alternative for the detection and recovery of *Salmonella* in these select gum commodities.

P1-116 Evaluation of Molecular *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium Assay Performance in Poultry Meat Samples

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Introduction: Thermo Scientific TaqMan Salmonella Triplex Assay with lysis is a PCR based test for the simultaneous detection and differentiation of *Salmonella* species, *Salmonella* Enteritidis and *Salmonella* Typhimurium in food and environmental samples. The TaqMan Salmonella Triplex Assay workflow includes a simple 15-minute sample lysis step followed by a 40-minute PCR run on Applied Biosystems 7500 Fast Real-Time PCR instrument.

Purpose: The purpose of the study was to verify performance of the *Salmonella* Triplex Assay with a range of different poultry samples and compare the results to those obtained with the DuPont BAX System Real-Time *Salmonella* Assay or bioMérieux VIDAS-UP *Salmonella* Assay at two major poultry meat manufacturers' laboratories in Brazil.

Methods: A total of 152 naturally contaminated poultry samples representing 13 different matrix types were tested at Poultry Meat Manufacturer 1 (PMM 1). Additionally, 12 poultry samples representing 2 matrix types were inoculated with *Salmonella* Enteritidis or *Salmonella* Typhimurium at Poultry Meat Manufacturer 2 (PMM 2). Three samples were inoculated with a competing background of either *Escherichia coli* or *Enterobacter aerogenes*. All samples analyzed with the *Salmonella* Triplex kit were enriched in Buffered Peptone Water at 37°C for 18 hours, lysed, tested and analyzed according to the protocol described for the TaqMan *Salmonella* Triplex Assay with lysis. The BAX or VIDAS-UP *Salmonella*analysis was performed according to the manufacturers' instructions.

Results: The Salmonella Triplex Assay method proved to be an accurate method. One hundred forty-nine of the 152 samples tested (98.0%) yielded comparable results with the BAX and VIDAS-UP methods at PMM 1 laboratory and 100% (12/12) of samples gave the correct results in the analysis conducted at PMM 2.

Significance: The study demonstrated that the TaqMan Salmonella Triplex Assay method offers a rapid, easy-to-use and reliable workflow for the detection of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium in poultry samples.

P1-117 An Eight-year Perspective on Analyst Proficiency in the Detection of Typical and Atypical *Salmonella*

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Introduction: Accurate detection of typical and atypical *Salmonella* is important to both food safety and epidemiological studies. Proficiency testing (PT) can be used as a tool to assess the *Salmonella* detection and differentiation capabilities of laboratory analysts.

Purpose: This study analyzes data collected from fifteen PT studies completed in the United States and Canada over the course of eight years. These studies were conducted in various food matrices and represent results obtained from city, state, federal, public health, and private laboratories.

Methods: Food and veterinary diagnostic samples were artificially inoculated with *Salmonella* serovars and confounding cultures ranging from 1-10^5 CFU/g to mimic actual samples. Participants were instructed to isolate, identify, and confirm *Salmonella* serovars utilizing routinely used methods. Food matrices studied included black pepper, lettuce, liquid eggs, infant formula, catfish, and veterinary diagnostic samples.

Results: Among 916 total participants, the passing rate was over 92% across fifteen *Salmonella* PT studies. Out of 72 analysts testing black pepper, 88% detected atypical *Salmonella* Heidelberg, while 100% of analysts were able to detect typical *Salmonella* serovars. In veterinary samples analyzed by 25 participants, 83% correctly detected *Salmonella* Typhimurium at 1 CFU/g and 76% correctly detected *Salmonella* Heidelberg at 1 CFU/g. Detection of *Salmonella* Typhimurium in bagged lettuce among 75 participants was 99% and 100% when inoculated at 10-20 CFU/g and 99% correct when inoculated at 110-180 CFU/g. The overall passing rate of *Salmonella* detection was 100% in bagged lettuce, black pepper, and liquid eggs; while average passing rate in veterinary diagnostic samples was 92% and 100%.

Significance: The studies summarized indicate analyst proficiency is robust in the detection of typical and atypical *Salmonella* serovars. Preparation of samples according to principals of ISO 22117 with inoculation levels near the limit of detection and confounding organisms could be used to identify the problematic areas of *Salmonella* detection in food.

P1-118 Efficacy of *Salmonella* Detection in Ground Beef and Cilantro by Five Commercially Available Tests

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Introduction: Being a commonly prevalent microorganism in various food items, *Salmonella* is frequently tested by the industry. Multiple tests for *Salmonella* detection are commercially available; however, little information is available that compares the performance of tests.

Purpose: To compare various methods to detect *Salmonella* in ground beef and cilantro.

Methods: Five methods were compared: Petrifilm *Salmonella* Express System (SALX) (3M); Molecular Detection Assay (MDS) (3M); BAX System (Dupont Qualicon); GeneDisc (PALL Corporation); and the gold standard from FDA and USDA (USDA MLG 4.08 for ground beef; Bacteriological Analytical Manual (BAM) for cilantro). Commercially-available cilantro and irradiated ground beef were used. Samples were inoculated with a five-strain *Salmonella enterica* cocktail, (Enteritidis, Newport, Typhimurium, Heidelberg, and Blockley). Protocols indicated by each method were followed. A total of 92 tests were conducted per method and sample type, divided into four groups of 23 each as follows: 23 un-inoculated samples and three groups of 23 each inoculated with the cocktail at different levels of *Salmonella* concentration (attachment of ca. 0.6, 1, and 2 log, respectively). Data was analyzed using the Generalized Linear Model (GLM) with the statistical software SAS 9.4.

Results: All methods were able to detect 100% of *Salmonella* from inoculated ground beef; statistical difference (P=0.0003) was found among methods only with un-inoculated samples, from which MDS, SALX, and BAX detected positives (60.87%, 40.00%, and 8.70%, respectively). Regarding cilantro, all methods detected *Salmonella* at the two highest levels of inoculation. SALX failed to detect Salmonella in 22% of the lowest inoculation level. No statistical difference (P>0.05) was found among methods. BAM, GENEDISC, MDS, and SALX detected positive samples from un-inoculated controls.

Significance: Comparing the efficacy of *Salmonella* detection by different tests, provides useful information to the food industry from an independent source.

P1-119 Microscopic and Cytometric Characterization of Salt- and Cold-filamented *Salmonella*

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Introduction: Conditions commonly used in food preservation, including low water activity and low temperature, are known to induce the formation of filaments in *Salmonella* spp. The practical impact of filamentation on our ability to capture, concentrate and/or detect these physiologically atypical cells is unknown. Further characterization of cold- or salt-filamented salmonellae is needed in order to more fully understand the unique physiological characteristics of filaments produced under different conditions.

Purpose: To define filament-inducing conditions and timeframes for several strains of *Salmonella*, to characterize and compare morphological and physiological features of *Salmonella* filamented under different conditions, and to develop a quantitative, flow cytometry (FCM)-based method for monitoring septation and division of filaments upon removal of salt- or cold-stress.

Methods: Five *Salmonella* strains (ATCC 14028, ATCC 13311, ATCC 13076 and two peanut butter outbreak-associated isolates) and an *E. coli* control (ATCC 25922) were grown under four filament-inducing conditions of salt- or cold-stress in liquid or solid media. Additional *Salmonella* controls (overnight growth in TSB and extended growth in TSB) were also prepared. The resulting cultures were characterized using fluorescence microscopy (FM) and FCM in conjunction with LIVE/DEAD staining. Time course FCM analysis was used to monitor differentiation of filaments into individual cells after removal of stress conditions.

Results: All *Salmonella* strains formed filaments in response to salt-stress (within 5-6 d) or cold-stress (within 10-16 d) and the extent of filamentation was examined and compared across strains and conditions using both FM and FCM. We found that post-stress transition from filaments to single cells could be monitored quantitatively over a period of 6 h using time course FCM.

Significance: This study provides single-cell approaches for physiological characterization of stress-filamented *Salmonella* populations that may help elucidate the practical impact of filamentation on detection of this pathogen in foods.

P1-120 Detectability of Salt- or Cold-filamented *Salmonella* Using Cultural and Molecular Techniques

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Introduction: Common stressors in food processing environments include exposure to high concentrations of salt or other solutes and low temperatures. While these conditions are known to induce filamentation in *Salmonella* spp., the practical impacts of filamentation detection of *Salmonella* are unknown. Further work is needed to determine the limits of existing cultural and molecular techniques in detecting filamented salmonellae.

Purpose: To evaluate the impact of stress-mediated filamentation on the utility of various cultural and molecular techniques for detection and characterization of *Salmonella*.

Methods: Five *Salmonella* strains, including two peanut butter outbreak-associated isolates and an *E. coli* control were grown under four filament-inducing conditions of salt- or cold-stress in liquid or solid media. Additional non-filamented *Salmonella* controls were also prepared. These cul-

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tures were characterized by 1) Comparative quantification by optical density, plating and qPCR, 2) Combined nucleic acid staining and Flow Cytometry (FCM), followed by qPCR of sorted populations, 3) Peptide nucleic acid-based fluorescence in situ hybridization (PNA-FISH) and 4) Reverse transcriptase-qPCR (RT-qPCR) for measuring flagellar gene expression.

Results: Comparison of plating and qPCR results revealed a disconnect between cultural and molecular methods, with qPCR yielding higher apparent quantification than plating for filamented *Salmonella*. Recovery of filaments on XLT-4 was lower than on TSA, suggesting lowered fitness or injury. Stress-filamented treatments grouped into four distinct subpopulations according to nucleic acid content, and qPCR on FCM-sorted subpopulations highlighted the impact of cellular heterogeneity on molecular testing results. Finally, filaments contained sufficient rRNA to allow detection by *Salmonella*-specific PNA-FISH, but transcription of flagellar genes (*fliC*, *fljB*) was dramatically decreased compared to controls.

Significance: Our results highlight both the utility and drawbacks of existing cultural and molecular methods for detection of stress-filamented salmonellae. We expect our work will inform the use of appropriate tools for detecting the presence of such filaments in foods.

P1-121 Validation of a Novel Secondary Enrichment Broth for Resuscitating Viable but Nonculturable (VBNC) *Salmonella* spp. in Environmental Samples

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Introduction: The survival capacity of *Salmonella* increases the probability of the contamination of agricultural commodities and their processing environments. Sampling food production and processing environments can be difficult, where bacteria may enter a non-viable state in order to protect themselves from environmental stress. Traditional culture methods are effective for food testing, but modifications need to take place in order to allow a more efficient recovery of stressed cells from the environment. Studies have shown that *Salmonella* is able to enter a VBNC state, regain culturability and cause disease once it has entered the intestinal tract of a host.

Purpose: The objective of this study was to evaluate the effectiveness of a modification of Rappaport Vassiliadis broth and compare it to the recommended secondary enrichment from regulatory agencies for the recovery of VBNC *Salmonella* cells in environmental samples.

Methods: Samples obtained from a sweet potato field fertilized with composted chicken litter (soil, *n*=28), and a catfish farming area (pond water and mud, *n*=40) were pre-enriched in Buffered Peptone Water, transferred to Rappaport Vassiliadis, Tetrathionate, and biochemically modified Rappaport Vassiliadis, incubated at 41.5°C and further analyzed using USDA/FSIS and BAX real-time PCR methods. Presumptive colonies separated on Brilliant Green Agar and Xylose Lysine Deoxycholate agar were confirmed by PCR.

Results: Detection rates for the soil samples were 43% on real time PCR, 54% on Rappaport Vassiliadis, 43% on Tetrathionate, and 61% on modified Rappaport Vassiliadis, and for pond water and mud these were 13% on real time PCR, 8% on Rappaport Vassiliadis, 8% on Tetrathionate, and 23% on modified Rappaport Vassiliadis.

Significance: Modified Rappaport Vassiliadis could be a good alternative enrichment broth to resuscitate VBNC cells that would go undetected with traditional methods and that could regain viability, contaminate foods, and cause foodborne outbreaks.

P1-122 Development of a Single-tube Multiplex Screening Assay for the Identification of Salmonella Heidelberg, *Salmonella* Enteritidis, *Salmonella* Typhimurium, and Salmonella spp. in the Poultry Environment

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Introduction: *Salmonella* serovars Heidelberg, Enteritidis, and Typhimurium (HET) have been identified as increasingly significant human outbreak pathogens originating from poultry. To meet the need for efficient testing of poultry environmental samples, BioControl Systems developed a multiplex real time PCR-based screening method, Assurance GDS *Salmonella* HET Tq, to simultaneously identify each of the three HET serovars, as well as generic *Salmonella* spp. in a single reaction tube. A rapid immuno-magnetic separation step (IMS) is incorporated in the assay.

Purpose: To evaluate inclusivity and exclusivity of the method and the ability to predict semi-quantitative levels of contamination of environmental samples.

Methods: Inclusivity of the Assurance GDS *Salmonella* HET Tq assay was evaluated with 12 isolates of *Salmonella* Heidelberg, 18 *Salmonella* Typhimurium, and 14 *Salmonella* Enteritidis. Additionally, 17 isolates of non-HET *Salmonella* were analyzed to test exclusivity of the HET channels, and simultaneously the inclusivity of the *Salmonella* spp. detection. Thirty non-*Salmonella* isolates were used to test exclusivity of the HET assay. Test strains were grown in non-selective broth, and diluted 10^{-4} or 10^{-2}, respectively, for inclusivity and exclusivity. For semi-quantitative testing, cultures of *Salmonella* Heidelberg, *Salmonella* Enteritidis, and *Salmonella* Typhimurium were grown in TT medium and serially diluted. A standard curve was generated by plotting cycle threshold (Ct) vs. CFU/ml.

Results: Inclusivity testing of 61 *Salmonella* spp. resulted in the correct determination of each culture. All 30 exclusivity strains were not detected. Semi-quantitative standard curves were linear from 10^4 to 10^8 CFU /ml (R^2 >0.95, *n*=60).

Significance: This single-tube multiplex screening method provides the poultry industry with a robust, efficient method to simultaneously identify three significant poultry-associated *Salmonella* serovars and *Salmonella* spp. in the poultry processing environment. The semi-quantitative characteristic of the assay may help assess contamination levels in poultry environmental samples.

P1-123 Comparison of Two Inoculation Methods for Detecting *Salmonella* in Fresh Leafy Greens and Fresh Herbs

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Introduction: Artificially inoculated food samples are used by FDA for microbial method development and validation. Our validation guidelines require all commodities to be inoculated at levels intended to give fractional results (25 to 75% positives), to permit statistical claims about the relative efficacy of treatments at the limit of detection (LOD) of one or both methods.

Purpose: To identify whether spot inoculation of individual analytical units or spray bulk inoculation of a large quantity of leafy greens and fresh herbs provides the most homogenous inoculation.

Methods: Ten (10) varieties of produce were used. For spot inoculation, 20 test portions, each containing 25 g of produce, were weighed out into sterile Whirl-Pak filter bags and inoculated with a single *Salmonella* serovar. For spray bulk inoculation, an amount of produce equivalent to at least twenty 25-g test portions was inoculated and mixed in a sterilized tote with a single *Salmonella* serovar. Both methods used an inoculation level of ~0.7 CFU/25 g. All inoculated samples were stored at 4°C for 2 to 3 days prior to analysis. On the day of analysis, we prepared 20 test portions (25 g) from

the bulk inoculated produce. Then all 40 test portions were preenriched separately in 225 ml modified buffer peptone water for 24 h at 35°C. The BAM culture method was followed thereafter, and qPCR was performed from 24 h and 48 h enriched cultures.

Results: Both inoculation methods generated fractional results; however, spot inoculation for individual units provided better fractional results: 44% positive portions were close to the ideal fraction (50% positive and 50% negative); spray bulk inoculation provided 33% positive portions. Spot inoculation was also much less laborious to perform than spray bulk inoculation.

Significance: Using the most effective inoculation method will significantly enhance the rate of successful experiment runs to support microbial method development and validation.

P1-124 Validation of the DuPont BAX System X5 for Detection of *Salmonella* spp. and *Escherichia coli* O157:H7 from Foods

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Introduction: *Salmonella* and *E. coli* O157:H7 are found in many food and environmental sources and can cause serious illness. Since isolation by culture can be a lengthy and labor intensive process, especially in the presence of competing flora, rapid simple methods for their detection are needed.

Purpose: This study evaluated inclusivity, exclusivity, and detection capabilities of the DuPont BAX System X5 PCR assays for *Salmonella* species and *E. coli* O157:H7 for detecting these pathogens in a variety of food types.

Methods: S*almonella* species (n=112 strains) and *E. coli* O157:H7 (n=45) inclusivity testing was performed at ~10^5 CFU/ml, while exclusivity testing (n=48 and n=46, respectively) was performed at ~10^8 CFU/ml.

For method effectiveness, 2 different foods (ground beef and romaine lettuce) for *Salmonella* species and 3 different foods (raw beef trim, romaine lettuce and red leaf lettuce) for *E. coli* O157:H7 were inoculated at fractional positive levels and evaluated using the BAX System X5 PCR assays. All samples were culture confirmed following either the FDA-BAM or USDA-FSIS MLG reference method.

Results: The collection of strains evaluated demonstrated 100% inclusivity for the target organisms. Testing using non-target strains for both assays demonstrated 100% exclusivity. For effectiveness testing, 240 PCR tests were performed across all matrix/media/enrichment/time conditions with a >98% concordance to culture confirmation. Analysis using the AOAC POD model indicated PCR and culture results were statistically indistinguishable.

Significance: This study indicates that the BAX® System X5 PCR assays for *Salmonella* and *E. coli* O157:H7 are a rapid and sensitive method for detecting these pathogens in food. Test kit results demonstrated no significant difference when compared with the reference culture methods.

P1-125 Title: Validation of RapidChek® *E. coli* O157 (including H7) and Select™ *Salmonella* Test Methods for Detection of *E. coli* O157:H7 and *Salmonella* species in Cannabis

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Introduction: *Escherichia coli* O157:H7 and *Salmonella* are important human pathogens linked to numerous food borne outbreaks due to the consumption of contaminated raw and processed food products. RapidChek *E. coli* O157 and SELECT *Salmonella* test systems can detect these pathogens in *cannabis*.

Purpose: The aim of these studies was to evaluate the performance of the *E. coli* and *Salmonella* test systems for the detection of *E. coli* O157:H7 and *Salmonella* spp. in *cannabis*.

Methods: For each study, *Cannabis* samples (1g) were inoculated with a low-level (3.0 cells per sample) inoculum of *E. coli* ATCC 35150 or with a low level (1.7 cells per sample) inoculum of *Salmonella* Typhimurium ATCC 14028. Twenty-two (22) samples, 20 low-level inoculated and 2 non-inoculated samples, were analyzed by each method.

Samples for the *E. coli* O157 test method were enriched in 9 mL of enrichment media and evaluated and screened with test strips at a total time of only 8 hours, and confirmed by using CT-SMAC agar, while samples for the *Salmonella* test method were enriched in 9 mL of primary media, transferred to secondary media, and evaluated with test strips at a total time of just 22 hours and confirmed using XLD agar.

Results: Both test method detected 20 out of 20 low-level inoculated *cannabis* samples. The negative controls were reported as negative with both methods. All presumptive test results confirmed via cultural confirmation on either CT-SMAC agar for *E. coli* O157 or XLD agar for *Salmonella*. Both methods exhibited 100% sensitivity and 100% specificity in these studies.

Significance: The *E. coli* test system detects low levels of *E. coli* O157 species in *cannabis* in as little as 8 hours and the *Salmonella* test system detects low levels of *Salmonella* spp. in *cannabis* in as little as 22-30 hours. RapidChek will provide method users with rapid, reliable, cost effective tools for monitoring and controlling *E. coli* O157 and *Salmonella* species in *cannabis*.

P1-126 Validation of RapidChek SELECT *Salmonella* Test System for Detecting Low Levels of *Salmonella* spp. in Cocoa Powder

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Introduction: Human salmonellosis continues to be a significant worldwide public health problem in many different raw and processed food products. RapidChek SELECT *Salmonella* test system can detect low levels of *Salmonella* spp. in cocoa powder.

Purpose: The aim of the study was to evaluate the performance of the *Salmonella* test system compared to the FDA BAM (Chapter 5 Salmonella) for the detection of *Salmonella* spp. in cocoa powder.

Methods: Cocoa powder samples (25g) were inoculated with a low level, (3 cells per sample) of *Salmonella* Enteritidis ATCC 13076. Twenty five samples, 20 low-level inoculated and 5 non-inoculated samples, were analyzed by both methods.

Samples for the test method were enriched in 225 mL of primary media, transferred to secondary media, and evaluated with test strips at a total time of just 22 hours and confirmed using HE and XLD agars. For the FDA BAM method, samples were enriched and incubated in skim milk supplemented with brilliant green solution, transferred to Tetrathionate broth and Rappaport-Vassiliadis broth, and struck to HE and XLD agars for a total of 66-78 hours.

Results: The test method detected 10 low-level inoculated cocoa powder samples, while the FDA BAM reference method detected 6 samples as positive. All samples were confirmed by the cultural method. There was no significant difference between the two test methods and equivalent performance was demonstrated. The overall Chi-square was 1.625 resulting in 167% relative accuracy, 100% sensitivity and 100% specificity.

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Significance: The *Salmonella* test system detects low levels of *Salmonella* spp. in cocoa powder in as little as 22 hours. RapidChek will provide users with a rapid, reliable, cost effective tool for monitoring and controlling *Salmonella* species in cocoa powder.

P1-127 Validation of RapidChek® Select™ *Salmonella* Test System for Detecting Low Levels of *Salmonella* species in Palm Oil

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Introduction: Human salmonellosis continues to be a significant worldwide public health problem in many different raw and processed food products. RapidChek SELECT *Salmonella* test system can detect low levels of *Salmonella* spp. in palm oil.

Purpose: The aim of the study was to evaluate the performance of the *Salmonella* test system compared to the FDA BAM (Chapter 5 Salmonella) for the detection of *Salmonella* spp. in palm oil.

Methods: Palm oil samples (25g) were inoculated with a low level, (2.5 cells per sample) of *Salmonella* Typhimurium ATCC 14028. Twenty five samples, 20 low-level inoculated and 5 non-inoculated samples, were analyzed by both methods.

Samples for the test method were enriched in 225 mL of primary media, transferred to secondary media, and evaluated with test strips at a total time of just 22 hours and confirmed using HE and XLD agars. For the FDA BAM method, samples were enriched and incubated in buffered peptone water (BPW), transferred to Tetrathionate broth and Rappaport-Vassiliadis broth, and struck to HE and XLD agars for a total of 66-78 hours.

Results: The test method detected 19 low-level inoculated palm oil samples, while the FDA BAM reference method detected 16 samples as positive. All samples were confirmed by the cultural method. There was no significant difference between the two test methods and equivalent performance was demonstrated. The overall Chi-square was 2.01 resulting in 119% relative accuracy, 100% sensitivity and 100% specificity.

Significance: The *Salmonella* test system detects low levels of *Salmonella* spp. in palm oil in as little as 22 hours. RapidChek will provide users with a rapid, reliable, cost effective tool for monitoring and controlling *Salmonella* species in palm oil.

P1-128 Comparative Evaluation of Sampling Devices and Enrichment Broths for Environmental Testing of *Listeria monocytogenes* on Different Food Processing Surfaces

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Introduction: Effective environmental testing procedures are critical for identifying source of contamination in food processing facilities; however, the current Food and Drug Administration Bacteriological Analytical Manual don't have validated methods for environment testing of *Listeria* spp.

Purpose: This research project was focused on the development and validation of testing procedures including sample collection and enrichment of *Listeria* spp. in food processing environments.

Methods: Five different surfaces, stainless steel, wood, rubber, glass and plastic were artificially inoculated with *Listeria* spp. and/or competing microflora. Four commercial brands of swabs and two commercial brands of sponges were comparatively evaluated for their ability to recover *Listeria* spp. from these surfaces. The enrichment scheme of half Fraser broth as pre-enrichment followed by Fraser broth as selective enrichment was compared to the enrichment scheme off our hours of enrichment in Buffered *Listeria* Enrichment Broth (BLEB) without antibiotics followed by 44 hours of enrichment in BLEB with antibiotics for their ability to enrich samples collected from these surfaces.

Results: The data show that there was no statistical difference (P>0.05) in the ability to collect environmental samples among all brands of swabs and between two brands of sponges. The two enrichment schemes appear to be equivalent for enriching *Listeria* spp. from artificially contaminated surfaces (P>0.05).

Significance: The sources of several listeriosis outbreaks were traced back to processing environment. Development and evaluation of environmental testing procedures can contribute to preventive control and outbreak investigation of *Listeria monocytogenes*.

P1-129 Comparison of an Alternative to the Standard *Salmonella* Whole Carcass Post-chill Test for Evaluation of First-processing Performance in Poultry Operations

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Introduction: The *Salmonella* carcass post-chill test (Postchill) is meant to measure how well a poultry processing establishment is able to minimize contamination, and the effectiveness of antimicrobial controls during first processing. Given more stringent standards for *Salmonella* in poultry, evaluation of tests that more accurately reflect first processing performance are needed.

Purpose: This study evaluated an alternative test (Staged) technique for post-chill *Salmonella,* comparing it to the standard USDA protocol.

Methods: In three poultry processing facilities, a carcass was aseptically separated into parts after conducting the standard USDA carcass post-chill test. The parts were placed into a bag and allowed to rest for 20 minutes. After this period, 400 ml of Buffered Peptone Water (BPW) was poured into the bag and agitated for one minute. The BPW was collected and sent for laboratory testing using the standard USDA *Salmonella* PCR protocol. Since both tests were conducted on the same carcass, this allowed use of McNemar's test to compare each test's ability to detect *Salmonella*.

Results: A total of 8,131 test pairs were examined, resulting in odds of 9.8 (P<2.2 X 10^{-16}) of a Staged test detecting a *Salmonella* positive when Postchill was negative (95% CI: 7.1, 13.8). Plant A (4,326 test pairs) exhibited an odds of 9.8 (P=<2.2. X 10^{-16}; 95% CI: 6.6, 15.2), Plant B (1,896 test pairs) an odds of 7.3 (P=3.2 X 10^{-8}; 95% CI: 3.1, 21.1), and Plant C (1,909 test pairs) an odds of 11.2 (P<2.2 x 10^{-16}; 95% CI: 5.9, 25.2) that Staged would detect a *Salmonella* positive when Postchill was negative.

Significance: When Postchill and Staged test results were different, the Staged test was significantly more likely to detect a *Salmonella* positive carcass. The Staged test is likely a more accurate measure of first-processing performance than the current test selected by USDA for verification.

P1-130 Prevalence and Conditions of Mechanical Tenderization and Enhancement of Beef at Independent Meat Retailers in North Carolina

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Introduction: In 2016 new USDA Food Safety and Inspection Services rules require raw or partially cooked beef products that have been mechanically tenderized or enhanced to be labeled as such. The tenderizing process increases the risk of pathogen internalization requiring different risk management steps during cooking. Information about the population and practices of those that are processing meat in this way is needed to create educational interventions targeting small independent meat retailers.

Purpose: The purpose of this study was to determine the prevalence of mechanical tenderization and enhancement of beef being prepared at independent meat retailers. For those independent meat retailers that were tenderizing or enhancing their beef products, storage parameters and practices were determined.

Methods: A list of meat markets was obtained from a local health department. All independent meat markets were visited where an in-person survey was conducted, consisting of questions regarding mechanical tenderization and enhancement and product storage parameters. Additionally the survey was delivered at other independent meat retailers, permitted as restaurants or food stands.

Results: Of the 29 independent meat markets listed on the Wake County local health department list, 8 were tenderizing beef at the store itself using either Hobarts or mallets. Of the additional meat retailers that were visited in Wake County outside of the original list, 3 entities were tenderizing beef products using either mallets or hand-held equipment. Two retailers were found vacuum tumbling beef products with added marinade.

Significance: Once the prevalence and parameters of these processes are defined, meat retailer managers and employees can be educated about food safety practices that could potentially reduce the risks surrounding these types of products.

P1-131 Food Safety Culture: State of the Art and Application in an Italian Experience

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Introduction: Euroservizi Impresa Srl (ESI), an Italian consulting company throughout Italy and northern EU has proposed its "Global Management System" or "ESI **Methods** " including the multiple and simultaneous management of food safety, safety on workplace and privacy code, to the Italian and Northern European food companies.

Purpose: With the introduction of HACCP and the inclusion of the concept of self-control, Italian and European food companies has gone from a control on the final product to a preventive control of the entire chain. Italian and European food companies are characterized by a small dimension. We will try to explain how in the EU Union characterized by micro enterprises, it was possible to adapt to a radical regulatory change.

Methods: By analyzing a sample of 2,000 companies, it is understood that the main obstacle to change was represented in 85% of the total by a lack of understanding of the new language used; 10% of companies who understood the law, did not know, how to face the new manifold costs; the remaining 5% could see the new rule as an imposition, a stretch made by the EU Union. The new regulations were transformed from simple sheets in black and white in color-rich designs manual that transformed the concepts in friendly characters.

Results: The new approach has been amazing. In just two years, 95% of companies has adapted easily to the new European regulations. The remaining 5% is divided into 60% of micro-businesses that have managed to change in another year's time, and 40% who underwent change and closed.

Significance: Resistance to change is a physiological human defense mechanism. The success of a behavior change is based into understand what needs to be done and why. A winning proposition, not only must reach the goal, but must take root and then sprout.

P1-132 Improper Food Safety Behaviors Exhibited by Celebrity Chefs Create Need for Intervention

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Introduction: Consumers obtain information about foodborne illness prevention from many sources, including television media. However, some of the techniques exhibited may not meet the guidelines proposed by food safety experts. With the growth of cooking shows on television, it is important to determine the possible influence these may have on consumer behaviors.

Purpose: This study assessed the food safety behaviors exhibited by celebrity chefs on a variety of cooking shows to see if they meet the recommendations made by government organizations.

Methods: Using a structured guide, celebrity chefs (n=24) were observed while preparing meat dishes on 100 episodes of television cooking shows. Behaviors were categorized and compared to standard recommendations. Numbers of positive and negative behaviors were calculated.

Results: Proper modeling of food safety behaviors was limited. Many incidences of errors were recorded. For example, although all chefs washed their hands at the beginning of cooking at least one dish, 88% did not wash (or were not shown washing) their hands after handling uncooked meat. This was compounded with many chefs who added food to dishes with their hands (79%) or ate while cooking (50%). Other poor behaviors included not using a thermometer (75%), using the same cutting board to prepare ready-to-eat items and uncooked meat (25%), touching hair (21%) and licking fingers (21%).

Significance: This study suggests that there is a need for improvement in demonstrated and communicated food safety behaviors among professional chefs. The behaviors modeled by the chefs could lead to incidences of foodborne illness, especially among those who mimic their behaviors at home. Public health professionals need to design techniques to mitigate the potential negative impact of the poorly modeled behaviors.

P1-133 Changes in Lighting Conditions May Negatively Impact Perception of Doneness of Cooked Turkey Patties

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Introduction: Undercooked poultry is a potential source of foodborne illnesses. It is recommended that a food thermometer be used to check the temperature of cooked meat; however, researchers report that most consumers do not use a thermometer, instead using visual appearance as a doneness indicator. Perception of visual appearance relies on many factors, including lighting conditions. The effects of the changes in home lighting options in recent years on perception of color and perhaps doneness of meat are unknown.

Purpose: This study evaluated the effect of lighting sources on consumer perception of doneness and likelihood to eat turkey patties cooked to different internal temperatures.

Methods: Consumers (n=104) viewed standardized photographs of turkey patties cooked to six different internal temperatures, rated the perceived level of doneness of the patties and how likely they were to eat a patty that looked like the picture. Samples were viewed under five different lighting sources: 60 watt incandescent, and halogen, compact fluorescent, LED, and daylight LED lights at nominally the same lumen output.

Results: Lighting source significantly changed the perception of doneness of the samples. Soft white LED and halogen lights increased the perception of doneness the most, with a higher percentage of viewers awarding "Just about right" scores to samples that were not at the recommended safe temperature. Several participants reported that they would eat the patties that were less done than "just about right." Perception of doneness and likelihood to eat showed a discrepancy that should be further evaluated.

Significance: Recent changes in lighting regulations can affect perceptions of meat color, making it more likely that consumers will view poultry patties as done when they have not reached the appropriate temperature. This lends strength to the message of using food thermometers as the only reliable method of determining doneness of meat patties.

P1-134 The Go Noroviral Experiment: An Interactive Teaching Tool for Modeling Person-to-Person Disease Transmission

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Introduction: Human noroviruses are the most common cause of foodborne illness, though they are also commonly transmitted via person-to-person contact, particularly in places where people gather.

Purpose: The purpose of this work was to produce a citizen science interactive 'game' to be performed at a large international food safety conference, where attendees would participate in a mock norovirus outbreak that stemmed from one 'infected' person.

Methods: The infection was visibly and physically transmitted to other conference attendees using individually-numbered buttons (0-500) representing the virus. The button numbers as well as other data provided by the participants were used in standard epidemiological analyses to track and measure the scope of the outbreak, which is presented here for the educational benefit of the participants.

Results: Two hundred and forty-eight of the 500 buttons that were distributed returned to the distribution site to be recorded (49.6% response rate), and 88 people were responsible for transmitting the mock virus. Approximately one in seven attendees could have been infected, and on average, an infected person spread the virus to 2.48 other people.

Significance: Based on successful proof of concept, this interactive tool can be used by schools, exhibitors, and other educational groups to illustrate person-to-person transmission of infectious agents and the common formats for displaying and interpreting epidemiological data. Since the outbreak evolved based on the actions of the participants, each iteration of the exercise would be unique.

P1-135 Effect of Multi-phase Educational and Motivational Intervention on Cleanliness of Surfaces in a Commercial Kitchen

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❖ ***Developing Scientist Competitor***

Introduction: Cleaning and sanitizing food contact surfaces is one way to prevent cross contamination, a risk factor for foodborne disease. However, according to a 2014 FDA trend analysis report, contaminated food surfaces and equipment remain a problem in foodservice operations, suggesting the need for targeted interventions.

Purpose: To determine the effect of a multi-phase, motivation-based educational intervention to improve the cleanliness of surfaces in a commercial kitchen.

Methods: The cleanliness of 9 objects (6 food contact (FC) and 3 non-food contact (NFC)) in one commercial kitchen were measured from June to August 2015 using adenosine triphosphate (ATP) (UXL100 Clean-Trace™ Surface ATP, 3M, Minnesota, USA) and protein (ALLTEC60 Clean-Trace™ Surface Protein, 3M) swabs. Two to three swabs (over ~4 cm^2 of the surface) were collected from each object over three major stages of the study: baseline, procedural validation, and post-intervention. Surfaces were classified as clean if ATP measures were <300 relative light units (RLU) for FC; <1,000 RLU for non-food contact surfaces; and <3 µg of protein for both types of surfaces. The educational intervention, conducted after the baseline and procedural validation stages, consisted of 3 phases with the following motivational approaches: (1) management buy in, (2) monitoring and feedback using ATP and protein surface measurements, and (3) rewards.

Results: At baseline, 75% (n=9) of FC surfaces and 100% (n=7) of NFC surfaces were not clean based on ATP data, and 100% of surfaces (n=19) were not clean based on protein data. Post-intervention measures showed that 9 FC and 4 NFC surfaces were significantly cleaner ($P<0.05$) based on ATP while 3 FC and 4 NFC surfaces were significantly improved ($P<0.05$) based on protein.

Significance: Select surfaces were significantly cleaner after workers were exposed to the educational and motivational intervention. Our three-phase intervention could be used as a model for future educational and motivational interventions targeting foodservice workers.

P1-136 Using Revised Bloom's Taxonomy to Develop a Knowledge-transfer Module about Noroviruses

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Introduction: Human noroviruses are the leading cause of gastroenteritis outbreaks worldwide. Education surrounding noroviruses is critical as key features of the viruses vary from those of bacterial pathogens. A 2013 survey suggested several important gaps in U.S. food safety professionals' knowledge of noroviruses suggesting the need for targeted education.

Purpose: Our goal was to develop a knowledge-transfer module that targets food safety professionals and addresses prevention and control of noroviruses.

Methods: Revised Bloom's Taxonomy (RBT), a learning taxonomy centered around alignment among learning objectives, content, and assessment, was identified as a best-practice, theoretical underpinning for module development. Per RBT, a curriculum development team (2 content developers, 2 expert reviewers, 1 curriculum designer) was formed. Then, a course blueprint that included competencies and objectives was established. Next, essential content needed to master course objectives was determined (25 team hours to complete) then expanded into a detailed storyboard including knowledge assessment (27 h). Finally, the storyboard was converted to final course format (65 h). Expert reviewers identified essential and accurate content and evaluated the cognitive accessibility of complex source material (5 h). The course platform was developed using Articulate Storyline 2.

Results: The development of one asynchronous 30-minute module took 122 team hours to complete, including expert review. The narrated final product included on-screen text, imagery (photographs, illustrations, animations), and interactive learning activities (4). The course knowledge assessment contained multiple choice and drag-and-drop items.

Significance: Using RBT as an approach to develop a knowledge transfer module was time intensive and required expert review. While resource intensive, RBT systematically aligns objectives, content and assessment to maximize the effect of the curriculum. Such a curriculum will facilitate public health professionals' competency to work effectively with their constituent groups. Food safety educators can use this descriptive research to guide their own efforts.

P1-137 Comparison of Listeriosis Risk Factors among Three 'At-risk' Consumer Groups: Pregnant Women, Older Adults and Chemotherapy Patients

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Introduction: Due to weakened immune function, listeriosis is particularly associated with 'at-risk' consumers, predominantly affecting pregnant-women, older-adults(aged≥60years)and people receiving chemotherapy. Sporadic incidence of listeriosis is frequently related to refrigerated ready-to-eat(RTE)food products stored/consumed in the domestic kitchen. Consequently, 'at-risk' consumers should implement safe food handling/storage practices, including time and temperature control of RTE-foods to reduce the risks associated with *L.monocytogenes*. Changes in UK epidemiology data indicate reduced pregnancy associated-cases and increased older-adult and chemotherapy associated-cases.

Purpose: Determination and comparison of these 'at-risk' consumers' food-safety reported behaviours and related cognitions may identify listeriosis risk factors and inform the development of targeted food-safety information. Such data is currently lacking in the UK.

Methods: Quantitative survey methods(online/paper-based)ascertained food-safety knowledge, self-reported practices and attitudes towards domestic food handling/storage. The study involved pregnant/post-partum(≤12 months)women(n=40), older-adults(≥60 years)(n=100)and chemotherapy-patients/family-caregivers(n=172).

Results: Awareness of recommended refrigeration temperatures were significantly more widespread($p<0.01$)among chemotherapy-patients/family-caregivers(57%) than pregnant-women(25%) and older-adults(13%); attitudes towards recommended refrigeration temperatures were significantly more negative among older-adults($p<0.001$). However, self-reported refrigeration practices were not significantly different ($p>0.05$), with the majority (58-65%) reporting temperatures would 'never' be checked. Older-adults were significantly less knowledgeable of 'use-by' dates, had more negative attitudes($p<0.001$) towards 'use-by' dates and were more likely to report consumption of foods beyond the 'use-by' date($p<0.005$). Although older-adults' attitudes were significantly more negative towards consuming RTE foods within two days of opening, no significant differences in knowledge and self-reported practices were determined, with majority of all 'at-risk' consumers(82-89%) reporting consumption beyond recommendations. Failing to adhere to recommended food storage practices may increase the risks associated with listeriosis among these 'at-risk' consumers.

Significance: Significant differences in knowledge, attitudes and self-reported practices between 'at-risk' consumers may indicate why reduced pregnancy associated-cases and increased older-adult and chemotherapy associated-cases are reported in the UK. Study findings may be utilised for the development of targeted food-safety education to reduce risks of listeriosis.

P1-138 The Use of a Consumer-orientated Approach to Design and Develop Food Safety Interventions for Chemotherapy Patients and Family Caregivers

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Introduction: Chemotherapy patients have an increased risk of foodborne illness due to immunosuppression, indeed the risk of listeriosis is reportedly five times greater to chemotherapy patients. Consequently, ensuring food safety at home is essential for patients/family caregivers. However, it is suggested that limited food safety information is available to chemotherapy patients/family caregivers in the UK and data on food safety practices during chemotherapy are particularly lacking.

Purpose: To establish current food safety practices and preferences to inform the development of targeted food safety interventions to aid patients/family caregivers to implement risk-reducing food safety practices.

Methods: A review of UK food-safety information, along with a consumer-orientated approach involving in-depth interviews, self-complete questionnaires and focus groups, allowed for the design, development and evaluation of a targeted food safety education strategy.

Results: A review of food-related information available to chemotherapy patients obtained from 42 of 141 NHS chemotherapy providers established many failed to highlight importance of food safety to prevent infection, considerable gaps exist and information varied between sources. In-depth interviews (n=15 patients/family-caregivers) determined food safety information during chemotherapy was considered to be inconsistent, insufficient and particularly sought after. Self-complete questionnaires (n=172 patients/family-caregivers) determined that despite increased awareness of the importance of food safety, malpractices were reported and perceived risks were underestimated, particularly among patients. During chemotherapy, information on 'keeping active'/'healthy eating' were significantly ($P<0.05$) more likely to be received than on 'food safety.' Focus groups (n=23 patients/family caregivers) enabled design, development and evaluation of food safety education interventions. It was recognized that to enable a sense of 'control' for food safety, risk-reducing behaviors not only needed to be recommended, but why they are important needed to be addressed.

Significance: This research project has informed the design, development and evaluation of targeted food safety interventions using a data driven consumer-orientated approach. This, alongside input from food safety experts has resulted in tailored food safety resources that may help to increase implementation of risk-reducing food safety behaviors for patients undergoing chemotherapy treatment, and thus reduce risk of foodborne illness.

P1-139 An Ethnographic Approach to Assessing Food Safety Culture at a Processing Company.

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Introduction: The importance of food safety culture throughout the food industry has emerged in recent years because of its impact on foodborne illnesses and food recall events. Whereas several studies have characterized the food safety culture of foodservice and food retail establishments, little work has been done to study food safety culture in manufacturing environments.

Purpose: The purpose of this study was to analyze the food safety culture of Company X, a national food processor and distributor with over 60 plants in the United States, before designing new food safety training programs.

Methods: Qualitative data collection methods were developed using the framework of an ethnographic study. A researcher made observations at six plants of Company X and conducted interviews of employees with the objective of learning about their food safety-related actions and the reasoning behind their actions.

Results: Content analysis was performed by two subject matter experts to organize observations into themes: authority figures have the strongest influence over food safety behaviors; written protocols and record-keeping tasks tend to reinforce proper food safety precautionary actions; lack of understanding of food safety principles leads to lack of compliance.

Significance: The information gathered from this ethnographic study provides insight into the weaknesses and strengths of the food safety culture of Company X, which will be applied to designing tailored food safety training programs. This study can be used a code of practice to study and improve food safety culture of other companies throughout the industry.

P1-140 The USDA's "Small Plant Help Desk"

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Introduction: USDA's Food Safety and Inspection Service provides a one-stop resource for all inquiries regarding food safety issues and related regulatory compliance: "The Small Plant Help Desk," a toll-free telephone hotline and email service. The primary audience is the more than 6,000 small and very small slaughter and processing establishments under federal inspection and the over 1,500 more under state inspection programs. These constituents represent more than 90% of the total number of meat and poultry production establishments in the United States. Furthermore, the Help Desk is utilized by all sectors including representatives from government, academia and the public.

Purpose: Presentation looks at the origin of the "Small Plant Help Desk," its role in ensuring food safety, and how the USDA is proactively assisting small business today.

Methods: The Help Desk services more than 2,000 phone and email contacts annually. This presentation analyzes the call volume over a several year period. Findings include: industry trends, demographics, topic type, methodology, and year-to-year timelines. The result offers a unique perspective of the industry.

Results: There is a direct correlation between the Agency's grassroots outreach participation in industry events, meetings and conventions and the traffic to the Small Plant Help Desk. In other words, Help Desk call volume correlates to the frequency of FSIS' presence at high-impact industry events such as the International Association of Food Protection convention and exhibition and other national and regional gatherings. The resource, once introduced, is utilized; often repeatedly.

Significance: Data indicate that outreach efforts to communicate FSIS' services and resources, combined with responsive and expert assistance from the Small Plant Help Desk improves and expands the Agency's interface with the small and very small establishment stakeholder audience and further ensures the safety and wholesomeness of the American food supply.

P1-141 Impact of Location and Type of Food Business on the Food Safety Inspection Grades and the Nature of Non-conformities

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Introduction: Dubai is a multicultural city with more than 15000 food establishments spread out in two main geographic areas, Old Dubai (Deira) & New Dubai (Bur Dubai). Food safety department of Dubai municipality conducts regulatory inspection in these establishments to ensure food safety. However degree of compliance in food establishment varies with types of the establishment and the location with higher food safety non-conformities noted in establishments in certain areas of Dubai.

Purpose: This study explores the link between the food safety grades, restaurant's location and the type of restaurants in Dubai.

Methods: Data for this study were retrieved from the GIAFS (General Inspections and Fines System) of the department for 200 samples of chain and independent restaurants located in different areas for the year 2015.

Results: Grades from the chain and independent restaurants were used for a comparison of food safety compliance between the restaurant types within the same location versus different locations. Based on the evaluation of pooled means, significant differences were observed in the mean value in the each types and location of the restaurants. Amongst the chain restaurants, being located at old Dubai the restaurants were found to be less hygienic with the mean value of 2.46 (based on the grades) compared to those located in new Dubai with the mean value of 3.02. The independent restaurant in Deira shows a similar trend as compared to the one in Burdubai with the mean value of 1.64 and 2.84, respectively.

Significance: The study indicates a possible link between the location of restaurants and the number of violations and the overall food safety culture in the establishment. The studies assist the regulatory authorities in planning new requirements such as specialized interventions, infrastructure improvement, training to improve the knowledge of the staffs of the restaurants located in old areas and independent restaurants types.

P1-142 Why a More Effective Food Safety Curriculum is Needed: An Online Survey Results from High School Student

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Introduction: Currently, most food safety education is delivered through reading materials, and does not addressing high school students learning motivators and barriers. The positive deviance food safety curriculum, a novel educational intervention, allows students to discuss their food handling behaviors under the direction of a trained moderator and decide to try recommended practices modeled by peers like themselves.

Purpose: The objective of the study is to evaluate the effectiveness of a food safety curriculum utilizing positive deviance to increase high school students' food safety knowledge and safe handling practices.

Methods: A pilot group of high school students (*n*=114) completed an 18-question on-line survey. The survey assessed safe food handling knowledge and personal hygiene based upon the Cook, Chill, Serve and Separate behaviors as well as attitudes towards participating in a food safety class.

Results: The results showed that there is a critical need for effective high school food safety curriculum. The student participants had a low correct rate of the safe food handling knowledge. Merely 52% understood refrigeration and freezing does not kill bacteria. However, more than 80% thought the safest way to know meat was cooked thoroughly was to check the color, an inadequate and potentially risky food handling practice which has been addressed by food safety programs over the years. When the students were asked about knowledge of "choose safe food," their answers were even more worrying. Only 23% of the students believed that pasteurization does not significantly reduce milk/juice's nutritional value. One third were aware that irradiation makes meat and poultry safer to eat.

Significance: Findings demonstrate that, high school students need more effective food safety education. Knowledge change and reported behavior as a result of the positive deviance curriculum will be evaluated. Plans are to expand this approach to a larger audience of high school students.

P1-143 Assessing the Need for the Food Hygiene Rating Scheme (FHRS): An Investigation into the Association between the Compulsory FHRS and Third-Party Accreditation/Certification in Food and Drink Manufacturing and Processing Businesses (FDMPB) in Wales, UK

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Introduction: Welsh FDMPBs were required by law to have a FHRS score on display before anywhere else in the UK. The FHRS is now established in Wales and is achieved by inspection of FDMPB facilities, conducted by an Environmental Health Officer (EHO). FDMPBs are often required to have third-party accreditation/certification by retailers and the foodservice. The main third-party accreditations recognized by the industry are British Retail Consortium (BRC) and Safe and Local Supplier Approval (SALSA). The UK Government is investigating third-party accreditation/certification can be used to benchmark hygiene standards in FDMPBs therefore negating the requirement for FHRS in certified businesses.

Purpose: An investigation into whether there is an association between FHRS and third-party accreditation/certification, to determine the requirement for an additional FHRS inspection.

Methods: Using a desk-based research approach, a comparison of all FDMPB in Wales that have achieved third-party accreditation/certification were analyzed against their FHRS score.

Results: Cumulatively, 17.3% of all FDMPB in Wales (*n*=121) have third-party accreditation (BRC-72.5%, SALSA-27.5%). Many of these businesses (41.9%) who have achieved third-party accreditation were also inspected for the mandatory FHRS (meat and dairy businesses excluded due to sector specific hygiene audits). More than half of the BRC certified FDMPBs achieved accreditation on an 'unannounced' audit. Analysis shows that there is an association between FHRS score and the BRC grade. FDMPBs with a grade-5 FHRS were associated ($P<0.05$) with the highest BRC grades.

Significance: Given the significant associations determined in this study, findings suggest that third-party accreditation/certification grade can be used to determine FDMPBs FHRS-scores. This would negate the requirement for FHRS inspections in third-party accredited/certified FDMPBs, thus reducing the burden of multiple-inspections on FDMPB and having a positive impact on EHO time allocation/workload. Furthermore, findings indicate a need to obtain an in-depth understanding of the drivers to obtain third-party accreditation/certification in Welsh FDMPBs.

P1-144 Development and Implementation of a Knowledge Transfer Mechanism to Facilitate Technical and Food Safety Support to Dairy Sector Small and Medium-sized Enterprises (SMEs) in Wales, UK

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Introduction: The dairy industry is of considerable economic and social significance in Wales with a reported total gross output of £417.6million. In recent years the industry has reportedly operated in a volatile environment resulting in milk price negotiations and an increased need for technical/food safety support, innovation and understanding to maintain and improve competitiveness and enable business growth.

Purpose: This study aimed to evaluate a food technology/safety knowledge transfer mechanism between academia, government and dairy sector SMEs as well as resultant impact of implementation upon operational and technical performance (2013-2015).

Methods: A process and impact evaluation of mini 'Knowledge-Innovation-Technology-Exchange' (KITE) dairy sector projects (*n*=48) involving SMEs, academic partners and food technology/science undergraduates was undertaken. Individual 'mini-project' reports were reviewed and analyzed using content analysis resulting in development of in-depth case studies (*n*=6). Engagement of the dairy industry with academic partners has been assessed according to SME needs and government priorities.

Results: Cumulatively, evaluative findings indicated that KITE 'mini-dairy-projects' provided an effective mechanism for linking dairy sector SMEs with food technology/safety academics and undergraduate students, thus facilitating knowledge transfer. Furthermore, the project enabled SMEs to engage with academics in businesses, workshops and conferences tailored to the needs of the dairy industry. Overall, 48% mini-projects focused upon technical needs, 31% market research, 17% new-product-development (NPD) and 4% sensory analysis/review studies. Case study examples included verification of hygiene systems and capability for removal of allergens such as nuts from equipment/surfaces; nutritional analysis of food product ingredients/assessment of compliance with the European Union Food Information for Consumer Regulation; development of new products within SME capabilities and market research reviews to facilitate identification of opportunities for NPD.

Significance: Implementation of KITE mini-projects demonstrated development and use of an innovative mechanism to facilitate successful engagement and focus between dairy SMEs, academics and undergraduates. Increased technical/food safety knowledge in SMEs enabled further development that is required for improved innovation and increased/sustainable business growth.

P1-145 Unmasking Seafood Mislabeling in U.S. Markets: DNA Barcoding as a Unique Technology for Food Authentication and Quality Control

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Introduction: Seafood mislabeling has been practiced both to meet market demand, as well as for economic gain, whereby cheaper species are substituted for high-value seafood items. Proper classification and identification of seafood is key in mitigating this mislabeling problem. Traditional methods of identifying species based on fish morphological characteristics are not always practical to unambiguously identify seafood products available for purchase.

Purpose: This study shows the usefulness of targeting the mitochondrial cytochrome oxidase I (COI) region to uniquely identify fish and seafood samples from across the United States.

Methods: The use of DNA sequencing for species identification is a reliable alternative to traditional methods like morphological determination, especially when food items have been subject to various forms of processing. We have used DNA extraction, PCR, and next generation sequencing to identify our samples and approximate the mislabeling frequency.

Results: Results show a rate of mislabeling among restaurants of 16.3% (28 out of 172 samples) in three regions of the United States. The rate of false labeling is essentially equivalent between these different regions.

Significance: The consequences of mislabeling include a variety of health hazards, such as allergic reactions, and these issues extend to social, religious, and economic realms as well. Studies such as this will ensure the quality of food and labels in the industry while informing people of the sorts of problems associated with different categories of food.

P1-146 Detection and Characterization of Multiple Enteric Viruses from Imported Individually Quick Frozen Breaded Oysters Associated with an Outbreak

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Introduction: Human noroviruses are the leading cause of non-bacterial shellfish-associated gastroenteritis. Detection of enteric viruses in foods can be a challenge due to the low levels and the inability to enrich or propagate.

Poster

Purpose: The objectives were to concentrate, extract, detect and characterize norovirus (GI, GII, and GIV), Aichivirus, adenovirus, enterovirus, hepatitis A (HAV) and hepatitis E viruses (HEV) in cooked and uncooked individually quick frozen (IQF) breaded oysters implicated in an outbreak. Male-specific coliphage (MSC), a municipal sewage indicator, was also enumerated.

Methods: IQF breaded oysters were analyzed, pre- and post-cooked, for norovirus genogroups (I, II, IV,) enteroviruses, adenoviruses, Aichivirus, HAV, and HEV. Ultracentrifugation was used for concentration of enteric viruses from implicated shellfish samples with murine norovirus (MNV) as an extraction control. Qiagen protocols and RT-qPCR/PCR assays were used for RNA/DNA extraction and detection. Conventional RT-PCR or RT-qPCR/qPCR and big-dye terminal sequencing was used to generate amplicons for characterization. MSC was enumerated by a double agar overlay technique.

Results: Levels of MSC in the uncooked oysters were 110 PFU/100 g. Norovirus GI, GII, GIV, enteroviruses, adenovirus, Aichivirus, and HEV were detected in uncooked oysters with average levels of 3129, 18296, 1835, 194, 877, 35780, 2160 RT-PCR/PCR units/100 g of digestive diverticula (DD), respectively. HAV was not detected. Levels persisted in post-cooked oysters for GI, GII, GIV, enteroviruses, adenovirus, Aichivirus, and HEV with averages of 824, 1687, <10, 234, 704, 6370, and 1429 RT-qPCR/qPCR units/100 g of DD, respectively. The average extraction efficiency of MNV was 68%. Sequence analysis for norovirus revealed ≥99% homology with GI.2, GI.5A, and GII.6.

Significance: This was the first reported incidence of norovirus associated illnesses due to consumption of fried breaded oysters. This is also the first instance of detection of norovirus genogroup IV in the US from shellfish implicated in an outbreak.

P1-147 Thermal Inactivation of Human Norovirus Surrogates in Oysters Homogenate

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❖ ***Developing Scientist Competitor***

Introduction: Human Norovirus (HNV) is considered most frequent causative agent of foodborne diseases in the United States. Raw and undercooked oysters are commonly involved in outbreaks caused by HNV. The FDA recommends that shucked oysters should be boiled for at least three minutes, but it is not clear this thermal treatment can inactivate HNV.

Purpose: The objective of this research is to evaluate whether this recommendation is sufficient to inactivate two HNV surrogates, Murine Norovirus (MNV-1) and Tulane virus (TV) in oyster meats.

Methods: Oyster homogenates were inoculated with ~5 log PFU/ml MNV-1 and ~3 log PFU/ml TV, respectively. The inoculated samples were treated in a circulating water bath maintained at 49, 54, 58, 63, 67°C for different durations. First-order model was used for calculating the D values of MNV-1 and TV at different temperatures. The oyster homogenates were also treated in a boiling water bath for 1, 2 and 3 min.

Results: *D*-values were 28.17 to 0.88 min for MNV-1, while they ranged from 18.18 to 1.56 min for TV in oyster homogenates treated at 49 to 67 °C. The kinetics demonstrated that TV was much more heat sensitive ($P<0.05$) compared with MNV-1, which might make it a less comparable virus surrogate as HNV in cooked oyster meats. One minute of boiling water had 0.73 and 1.22 log reduction for MNV-1 and TV, respectively, while 2 minutes of the treatment produced 3.64 log reduction for MNV-1 and below detect limitation (1 log PFU/ml) for TV. After 3 minutes of boiling, the titer of MNV-1 also dropped <1 log PFU/ml.

Significance: MNV-1 is more heat resistant than TV in oyster homogenates. Boiling the oyster meats for 3 minutes may be effective to inactivate HNV to below 1 log PFU/ml.

P1-148 Novel *Vibrio* Detection Method for Species and Toxigenicity Genes Identification Using Real-time PCR

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Introduction: *Vibrio parahaemolyticus, Vibrio vulnificus* and *Vibrio cholerae* are known to be potential waterborne contaminants of seafood and cause severe health problems worldwide. Traditional methods for the detection are time consuming and error prone, while real-time PCR can be done in less than 24 hours with a high specificity and sensitivity.

Purpose: The target was to design a real-time PCR assay, that can discriminate between *V. parahaemolyticus, V. vulnificus* and *V.* cholerae, and simultaneously detects and individually identifies the pathogenicity factors ctx, tdh, trh1 and trh2 by melting curve analysis in just one single reaction using sequence specific 5`Nuclease-probes. For convenience, the assay must be lyophilized.

Methods: Specificity (inclusivity/exclusivity) was tested with DNA extracts. Sample matrix compatibility, sensitivity and viability PCR were tested with genomic DNA and spiked samples.

Results: By using novel targets, false-positive and false-negative results, known from other methods using targets like, e.g., tlh or hlyA, are avoided. A total of 149 strains (74 *V. parahaemolyticus*, 26 *V. vulnificus* and 49 *V. cholerae*) were tested for inclusivity: With 100% specificity (inclusivity/exclusivity) for the detection of species and pathogenicity factors, the assay is superior to other methods for *Vibrio* detection. There were no false-positive results for all 73 tested samples of 54 closely related species and bacteria of the same habitat. The sensitivity of the **food**proof® Vibrio Detection LyoKit is 1 genomic equivalent (GE) per reaction for species detection and 10 – 25 GE per reaction for toxin detection. The assay is compatible with all 21 tested raw and processed seafood matrices like whole squid, raw oysters or smoked salmon. The sample preparation includes a live/dead discrimination by using Reagent D, which efficiently removes DNA of at least 10^3 CFU/ml dead *Vibrio*.

Significance: One hundred percent specificity and high sensitivity meets the demands of testing laboratories. As seafood often is contaminated with dead cells of *Vibrio* spp., Reagent D treatment prevents false-positive results which may be encountered with other PCR methods.

P1-149 Impact of Near-neutral Electrolyzed Oxidizing Water on *Vibrio* spp. in Eastern Oyster (*Crassostrea virginica*)

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Introduction: As a major group of pathogen to seafood-related gastrointestinal infections in the United States, *Vibrio* spp. is considered to be highly correlated to the safety of raw oyster consumption. Near-neutral electrolyzed oxidizing (NEO) water has been proved to be one effective method to control foodborne pathogens.

Purpose: To evaluate the antimicrobial efficacy of NEO water on *Vibrio* spp. in raw oysters

Methods: Fresh Eastern oysters were harvested and transported to the Laboratory on ice. Upon arrival at the lab, oysters were kept in 3.5% artificial seawater for a short period of depuration. NEO water (pH: 6.75; oxidation-reduction potential (ORP): 820.2; free chlorine: 98 ppm) was prepared by the food safety engineering lab at University of Georgia and tap water was used to make NEO water dilutions. Full NEO water, 1:1 and 1:4 dilution between NEO water and tab water were established as 3 treatments. The pH, ORP, and free chlorine were measured at 0, 3, 6, 12, and 24 h. Population of *Vibrio* spp. in oyster was determined by thiosulfate citrate bile salts sucrose (TCBS) agar plating at 0, 2, and 5 h.

Results: The result shows that free chlorine reduced significantly ($p<0.05$) by the preservation of oyster after 3 hours while free chlorine of NEO water without oyster remained stable. Significant difference ($P<0.05$) was also observed in ORP and pH value in NEO water with oyster in all three treatments and both parameters were changing dramatically ($p<0.05$) when free chlorine value dropped near 0. While *Vibrio* spp. population remained stable among all sampling points, significant difference (about 1 log)($P<0.05$) was detected between 2 h and 5 h in treatment of 1:4 diluted NEO as free chlorine dropped to 0 after 3 hours.

Significance: Result demonstrates the potential of using NEO water to control *Vibrio* spp. in oysters during depuration.

P1-150 Modeling of the Cross-contamination of *Vibrio parahaemolyticus* in Shrimp Peeling Process

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Introduction: *Vibrio parahaemolyticus* has been recognized as the leading cause of seafood-borne illness all over the world. The cross-contamination of *Vibrio parahaemolyticus* could be caused by gloves in the shrimp peeling process, which is a critical control point for ensuring the safety of peeled shrimps. However, the bacterial transfer due to the gloves in shrimp peeling process has not been studied well.

Purpose: The purpose of this study was to investigate the cross-contamination of *Vibrio parahaemolyticus* due to gloves and develop a mathematic model for describing the bacterial transfer in shrimp peeling process.

Methods: A pair of clean gloves was used to peel fifteen shrimps consecutively. The first shrimp was inoculated with three-strain cocktail *Vibrio parahaemolyticus* and the other fourteen shrimps were sterilized. Three inoculation levels of 6, 7 and 8 log CFU/ml were considered in this study. Bacterial concentration of each shrimp was investigated after peeling. A Weibull model was developed for describing the bacterial transfer in the peeling process, and ANOVA was used to test the significance and variance of the model.

Results: The subsequent bacterial transfers on non-inoculated shrimps from the contaminated gloves were found in all shrimps. A maximum of 5 log CFU/g of *Vibrio parahaemolyticus* could be transferred via gloves. The transfer took place at two distinguishable rates. The initial steeper slope at first seven shrimps, which demonstrated a fast bacterial transfer, is followed by a persistent low bacterial populations recovered from the last eight shrimps, forming a smooth tail of data points. Statistical indices (R^2=0.99; $P<0.0001$; RMSE=0.00034 and SEP=14%) indicated the satisfactory performance of the Weibull model.

Significance: The results of the study provide the information on cross contamination in shrimp processing plant and the constructed model could be a useful tool for the risk assessment during shrimp peeling process.

P1-151 Single Laboratory Validation of MPN-Real-time PCR Methods for Enumeration of Total and Pathogenic (*tdh+/trh+*) *Vibrio parahaemolyticus* in Oysters

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Introduction: *Vibrio parahaemolyticus* (*Vp*) is a leading cause of seafood-borne bacterial infections in the United States and other areas of the world. The primary route of *Vp* infections is through the consumption of raw or under cooked oysters.

Purpose: Currently, approved regulatory methods for *Vp* either lack quantitative capability, or rely on biochemical identification. As *Vp* is a ubiquitous organism, quantitation is increasingly important and biochemical identification has been found unreliable for these bacteria isolated from food and environmental samples. The intent of this study was to validate two MPN-real-time PCR methods: one for enumeration of total *Vp* (*tlh+*) and one for pathogenic Vp (*tdh+/trh+*).

Methods: Oysters processed to reduce vibrio levels were homogenized by standard procedures. Aliquots of the homogenates were spiked with known concentrations of *tdh+/trh+ Vp* strains. Appropriate dilutions of strains were spread plated in triplicate on TSA to determine spike levels. Spiked homogenates were used to inoculate a standard 3-tube MPN in APW. After overnight incubation, aliquots of turbid MPN tubes were tested for the presence of total or *tdh+/trh+ Vp*by real-time PCR on the Cepheid SmartCycler II and AB 7500 Fast platforms.

Results: The PCR portions of the methods were determined to have 100% inclusivity and exclusivity. Differences between the spike level and MPN-PCR values of the test methods were not statistically significant ($P>0.35$). Recovery of the test methods was determined to be between 100-102%. A significant correlation ($P<0.001$) was observed between spike levels and MPN-PCR values, across a range of concentrations (0.15-6.74 log *Vp*/g), with correlation coefficients between 0.97-0.98.

Significance: The results of this study demonstrate the validity of data obtained using these MPN-real-time PCR methods for enumeration of total and pathogenic *Vp* in oysters. Based on this study, these methods have been accepted for regulatory use by the National Shellfish Sanitation Program.

P1-152 Profile and Contributing Factors of *Vibrio parahaemolyticus* in Seafood Marketed in Shanghai, China

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Introduction: In order to devise intervention strategies to reduce the incidence of *Vibrio parahaemolyticus* outbreaks, it is necessary to identify the contamination rates and levels of *V. parahaemolyticus* in seafood and the contributing factors.

Purpose: The aim of this study was to profile the presence of *V. parahaemolyticus* in seafood obtained from the market in Shanghai, China, identify the seafood item most susceptible to contamination of *V. parahaemolyticus*, and determine whether the post-harvest handling affecting the prevalence and levels of this pathogen.

Methods: Seafood samples (shrimp, crab, scallop, oyster, and mussel) were collected at sea wharf and retail stores in Shanghai from July to December of 2015. Each sample (25 g) was incubated at 37°C for 8 h for most probable number analysis. *V. parahaemolyticus* was identified by streaking samples from MPN tubes on thiosulfate-citrate-bile salts-sucrose agar, followed by on CHROMagar Vibrio agar, and confirmed with real-time RT-PCR targeting three hemolytic genes, *tlh*, *tdh* and *trh*.

Results: A total of 35 samples were collected at sea wharf and 32 samples tested positive for *V. parahaemolyticus* (prevalence rate of 91.4%) with levels as high as 2.2 log CFU/g, whereas the prevalence rate was 95.8% (91/95) with levels as high as 3.4 log CFU/g for samples collected at the marketplace. Among the variety of seafood, shrimp had the highest prevalence rate and level of *V. parahaemolyticus*. The highest prevalence rate was 100% in

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samples collected in August, which was the warmest month in Shanghai area. Results show that shrimps are of a particular concern of *V. parahaemolyticus* contamination and the post-harvest practices increase the prevalence rate and level of *V. parahaemolyticus* in seafood marketed in Shanghai.

Significance: The finding suggests that improved post-harvest hygiene practices and proper refrigeration are critical in reducing *V. parahaemolyticus* hazards in seafood.

P1-153 Yellowfin and Albacore Tuna Microbiomes: Using Metagenomics to Improve Our Understanding of Scombrotoxin Fish Poisoning

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Introduction: Scombrotoxin fish poisoning (SFP) is the most common finfish-related illness in the US. To design effective mitigation strategies for SFP, it is necessary to understand the composition and distribution of scombrotoxin-forming microbial species associated with fish microbiomes.

Purpose: The objective of this study was to provide a baseline description of culture independent and culture dependent microflora associated with two commercially important and widely consumed fish; yellowfin and albacore tuna. A specific goal was the analysis of the anatomical incidence of histamine-producing bacterial species and co-occurring microbial community structure and diversity.

Methods: DNA was purified from gills, skin, and anal vent swabs of freshly caught yellowfin (*n*=3) and albacore (*n*=6) tuna. 16S rRNA gene amplicons were sequenced using the Illumina MiSeq V3 platform. Data were analyzed using the QIIME pipeline (Quantitative Insights Into Microbial Ecology). Enriched swab samples [48h at 25°C, Marine Broth (MB)+1% histidine] were analyzed for the presence of the histidine decarboxylase (*hdc*) gene using qPCR.

Results: Principal component analyses of 16S rRNA gene amplicons showed distinct taxonomic profiles for the three sampling locations: gills, skin, and anal vent. The most abundant class/order/family in all sampling locations was Gammaproteobacteria/Vibrionales/Vibrionaceae. *Photobacterium* was the most abundant genus (0.9%) observed in anal vent whereas only 0.1% of the OTUs from gills and skin were *Photobacterium* spp. Other important histamine-producing bacteria (HPB) such as *Citrobacter*, *Enterobacter,* and *Morganella* spp. comprised a small fraction of the observed OTUs at all sampling locations or 2-9 x 10^6, 0.1-5.8 x 10^6, and 0.4-1.1 x 10^6%, respectively. The *hdc* gene was only detected in anal vent enrichment samples from three albacore and two yellowfin tuna.

Significance: These data will be used to refine mitigation and detection strategies to control *Photobacterium* spp. and other histamine-producing bacteria in tuna and other fish species associated with SFP.

P1-154 Thermal Resistance of the Histidine Decarboxylase Enzymes from High Histamine-producing Bacteria

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Introduction: Precooking of tuna is a potential critical control point (CCP) in commercial manufacturing of canned tuna. An understanding of the thermal properties of histamine-producing bacteria (HPB) and histidine decarboxylase (HDC) enzymes is required to effectively evaluate the utility of this CCP. The thermal properties of HPB have been determined, but the thermal resistance of the HDC enzymes is unknown.

Purpose: The purpose of this study was to determine the *D*- and *z*-values of selected HDC enzymes in order to evaluate the CCP of precooking during the canning process and provide scientific data to support future FDA guidelines.

Methods: Histidine decarboxylase (*hdc*) genes from three strains each of *Morganella morganii*, *Enterobacter aerogenes, Raoultella planticola*, and *Photobacterium damselae* were cloned, expressed, and purified using the Champion pET Directional TOPO Expression System, pET100 cloning vector, and HisPur Cobalt resin. The heat resistance of all enzymes was compared at 50°C, and the *D*- and *z*-values from one strain of each HPB were determined from 50-60°C. To evaluate heat inactivation during canned tuna processing, tuna tissue was inoculated with HDCs and heated to 60°C.

Results: There was no statistical difference in *D*-values at 50°C within HPB species (*P*=0.061-0.117). The HDC from *E. aerogenes* had the highest *D*-value at all temperatures. The ranges of *D*-values for the HDC enzymes from *M. morganii*, *E. aerogenes*, *R. planticola*, and *P. damselae* were 1.6-4.1, 1.6-6.3, 1.9-4.3, and 1.6-2.9 min at temperatures ranging from 50-60°C, respectively. The *z*-values for *M. morganii*, *E. aerogenes*, *R. planticola*, and *P. damselae* were 19.2, 18.0, 22.0, and 13.3 min, respectively. The HDCs from all HPB except *E. aerogenes* showed no significant activity after heat treatment to 60°C.

Significance: The data generated in this study will help refine current guidelines for thermal destruction of the HDC enzyme.

P1-155 Effects of Vinegar Powder and Storage Temperature on *Morganella morganii* Growth and Histamine Production in Tuna Salad

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Introduction: Scombrotoxin (histamine) fish poisoning is associated with consumption of scombroid fish species such as tuna. Commercial tuna salad can present a food safety hazard when contaminated with histamine-producing bacteria (HPB) (e.g., *Morganella morganii*; Mm) from food handlers, processing equipment, or added ingredients, and combined with temperature abuse during processing/storage.

Purpose: The objective of this study was to determine the effects of natural preservatives, added ingredients, and temperature on growth and histamine (Hst) production by Mm in tuna salad preparations.

Methods: Ten (Hst analysis) and twenty-five (MPN-PCR) g of plain tuna salad (TS) and tuna salad with sanitized celery and onion (C/O) were inoculated with a three-strain cocktail of Mm (2 log CFU/g) that were previously isolated from raw scombroid fish. DV, a natural buffered vinegar powder (WTI, Inc., Jefferson, GA), was added to treated preparations at a concentration of 2% by weight; untreated preparations were used as controls. Samples were stored at 4° and 10°C for 4 weeks and 25°C for 3 days. HPB (Mm) were enumerated by MPN-PCR; Hst was determined fluorometrically.

Results: Mm did not grow at 4°C. At 10°C, Mm numbers ranged from 4 log MPN/g (307 ppm Hst) in TS to 8.3 log MPN/g (1164 ppm Hst) in C/O after 4 weeks. At 25°C, Mm numbers were 8.9 log MPN/g in both TS (1855 ppm Hst) and C/O (1589 ppm Hst) after 3 days. DV inhibited growth of Mm in TS and C/O at 10° (1.8 log MPN/g after 4 weeks) and 25°C (2.3 log MPN/g after 3 days).

Significance: We established that storage of TS and C/O at 4°C and addition of DV to TS and C/O inhibit Mm growth and Hst production that can result from temperature abuse of prepared tuna salad.

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P1-156 The Effect of Water Temperature on Bacteriophage MS-2 Persistence within Live Oysters (*C. virginica*)

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Introduction: Shellfish-borne viruses (i.e., noroviruses) are a challenging problem for raw oyster producers and consumers. Male-specific (MS) bacteriophages are considered better indicators of fecal impact and potential virus contamination than *E. coli*. As such, they are being evaluated by regulatory agencies as a means to access and improve the sanitary quality of raw shellfish.

Purpose: To characterize the ability of viable bacteriophage to persist within oysters as a function of time and holding temperature.

Methods: Oysters were individually exposed to 10^9 pfu of MS-2 bacteriophage in 500 ml of seawater at 15.5°C for 48 h followed by maintaining oysters within at 7.5, 15.5 or 24°C for up to 6 weeks in 40-liter aquaria containing approximately 25 liters of 28 ppt seawater. Oysters were evaluated for the presence of viable MS-2 bacteriophage within their meats weekly for 6 weeks.

Results: Log reduction rates of MS-2 bacteriophage within oysters were substantially influenced by water temperature. Log reductions observed 3 weeks post contamination for oysters were 2.33, 2.90, and 4.55 (N=2; *n*=6) held at 7.5, 15.5 and 24°C, respectively. After 6 weeks, observed log reductions were 3.56, 3.99, and non-detected (>5.99) for oysters held at 7.5, 15.5 and 24°C, respectively.

Significance: Results indicate that water temperature can play an extensive role in retention of MS-2 bacteriophage in seawater. Water temperatures should be taken into account by regulatory agencies when making management decisions or growing area classifications based on MS bacteriophage.

P1-157 Synergistic Antimicrobial Effect of Carvacrol and Zinc Oxide Nanoparticles against *Campylobacter jejuni*

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Introduction: Every year 8% Canadians are infected by *Campylobacter* spp., which is one the leading foodborne pathogens worldwide. *Campylobacter jejuni* is normally identified to be the most prominent isolates from human infections and causative agent of campylobacteriosis. According to Canadian Integrated Program of Antimicrobial Resistance, there is an increasing trend of *C. jejuni* isolated from humans and poultry products that are resistant to common antibiotic treatments.

Purpose: The purpose of this research is to determine the synergistic antimicrobial effect of carvacrol and zinc oxide nanoparticles (ZnO NPs) against *C. jejuni* as an alternative of antibiotic treatments.

Methods: Antimicrobial susceptibility of carvacrol, ZnO NPs, and a combination of both agents were tested against *C. jejuni* cocktail (a mixture of three strains). Macrobroth dilution broth adopted from Clinical and Laboratory Standard Institute guideline was conducted as a method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each antimicrobial treatment.

Results: MIC range of carvacrol was 13.5 µg/ml to 27 µg/ml. MBC of carvacrol (27 µg/ml) was effective to achieve 4.83 ± 0.11 log reduction (*P*<0.05) after 4 hours of treatment at 37°C. MIC range of ZnO NPs was 12.5 µg/ml to 25 µg/ml. MBC of ZnO NPs (25 µg/ml) was effective to achieve 4.81 ± 0.71 log reduction (*P*<0.05) after 4 hours of treatment at 37°C. Individual treatment of carvacrol (13.5 µg/ml) or ZnO NPs (12.5 µg/ml) did not show a bactericidal effect. However, a combination of 13.5 µg/ml of carvacrol and 12.5 µg/ml of ZnO NPs was effective to show a synergistic antimicrobial effect with 4.88 ± 0.08 log CFU/ml reduction (*P*<0.05) after 8 hours of treatment at 37°C.

Significance: These results suggest that carvacrol and ZnO NPs may potentially work synergistically to inactivate *C. jejuni.* Further research should be conducted to study the antimicrobial mechanism of this treatment.

P1-158 Rapid, Robust, Inexpensive Silver-iron Smart Nanomaterials for Killing Bacterial Pathogens

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Introduction: The emergence of drug resistant microorganisms has made the search for new effective antimicrobials inevitable. Nanoparticles, with their unique physico-chemical properties, are the most promising next generation therapeutics. The antimicrobial activities of silver ions are well known, however limited information is available on the effects of silver iron-nanoparticles (Ag-Fe NPs) on foodborne pathogens.

Purpose: In this study, we synthesized a series of silver-iron nanoparticles via co-precipitation method and evaluated their antibacterial activity against pathogens commonly associated with food and water borne infections.

Methods: Different combinations of silver- iron nanomaterials (Ag-Fe NPs) were synthesized via co-precipitation method and characterized by Powder X-ray Diffraction (PXRD), Fourier Transform Infrared (FTIR) Spectroscopy, Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), UV spectroscopy and magnetic studies. Microplate broth-dilution assays alongwith CFU plate count, vital staining and epi-fluorescence microscopy, were used to perform time-kinetics, MIC, recycling studies and *in situ* killing of bacteria within food matrices. Bacterial interactions with NPs were further confirmed by TEM.

Results: The synthetic Ag-Fe NPs were super-paramagnetic, ~ 30 nm in size, spherical with protruding ends. Short time exposure of bacterial cultures to NPs captured viable bacteria within 2 minutes while extended incubation exhibited a broad spectrum of antimicrobial activity against both gram-positive and gram-negative bacteria with MIC_{90} at 60 and 30 minutes of incubation, respectively. The MIC_{90} of Ag-Fe NPs against *B. subtilis* and *E. coli* was found to be 2.4 and 20 µg/ml, respectively. *In situ* killing by Ag-Fe NPs in water, milk and juice samples showed 97%, 49% and 4% killing of *B. subtilis* and *E. coli,* respectively. Recycling of Ag-Fe NP over 3 subsequent rounds retained the antimicrobial activity.

Significance: These studies suggest Ag-Fe NPs as a promising template for designing novel antibacterial agents that rapidly and repeatedly capture and kill pathogens from biological matrices at low concentrations.

P1-159 Characterization of Methicillin-resistant *Staphylococcus aureus* Isolated from Bovine Mastitic Raw Milk in Korea

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Introduction: Methicillin-resistant *S. aureus* (MRSA) has been constantly isolated from bovine mastitic milk. The presence of MRSA in milk poses a consistent concern of public health.

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Purpose: The current study was designed to investigate the frequency of MRSA isolations from raw milk in Korea, and characterize the patterns of antimicrobial resistance, virulence and genotype of the MRSA.

Methods: A total of 1,222 raw milk samples were collected from 47 dairy farms in Gyeonggi province during 2011 to 2012, and 643 milk samples were determines as mastitic milk based on the somatic cell counts. MRSA were isolated from mastitic milk and characterized for the profile of antimicrobial susceptibility. The presence of staphylococcal enterotoxins (SE) were screened by target specific PCR and the genetic profiles were determined by pulsed-field gel electrophoresis (PFGE), *Staphylococcus* protein A (*spa*) typing, and the SCC*mec*typing.

Results: Of 165 *S. aureus* isolated from mastitic milk samples, 23 S. aureus (13.9%) were determined as MRSA based on the detection of *mecA* gene, and all the MRSA isolates showed multi-drug resistance to more than 3 different antimicrobials tested. The most frequently detected SE gene profile was *seg*, *sel*, *sek*, *sem*, *sen* and *seo* genes (20 isolates, 87%). PFGE analysis showed the 23 MRSA isolated were grouped to 4 different PFGE types, and molecular typing revealed there were 3 kinds of SCC*mec-spa*types; IVa-t148 (n=11, 47.8%), IVa-t324 (*n*=9, 39.1%), II-t002 (*n*=3, 13.0%).

Significance: Previous studies and the current results indicate that the isolation rate of MRSA from raw milk has consistently increased in Korea. Since MRSA in raw milk is a serious threat to the public health, an effective monitoring and control program should be implemented to reduce the contamination.

P1-160 Investigation of Virulence Potential and Antimicrobial Resistance of Bacteriocinogenic Lactic Acid Bacteria Obtained from Homemade Cheese

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Introduction: Bacteriocinogenic strains are interesting as starter cultures to improve the safety and quality of the food products. However, to be safely applied, the strains must be free of risk to consumers. In this way, investigating the safety of isolates with bacteriocinogenic potential is important to ensure the human health.

Purpose: The aim of this study was to verify the presence of virulence factors, biogenic amines production, antimicrobial resistance and the presence of encoding genes of such virulence factors.

Methods: *E. hirae* ST65ACC and *P. pentosaceus* ST57ACC isolated from homemade cheese made with raw milk were subjected to PCR reactions to identify 52 genes related to antimicrobial resistance and virulence activity. Phenotypic methods were also used to identify biogenic amine production (tyramine, histamine, putrescine and cadaverine), virulence factors (gelatinase, hemolysis, lipase, DNAse,) and resistance to 12 antimicrobials (ampicillin, penicillin G, oxacillin, clindamycin, erythromycin, gentamicin, imipenem, rifampicin, chloramphenicol, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin).

Results: The tested isolates presented distinct combinations of antimicrobial resistance genes, but not necessarily the expression of such factors. For *P. pentosaceus* ST57ACC, eight genes were present, related to resistance to aminoglycosides, erythromycin and vancomycin, while in phenotypic tests, such isolate was resistant to vancomycin, oxacillin and trimethoprim/sulfamethoxazole. For *E. hirae* ST65ACC, only five genes, related to resistance to tetracycline, vancomycin and streptogramin were positive, and also phenotypic resistant to vancomycin, oxacillin and trimethoprim/sulfamethoxazole. None of the isolates presented production of biogenic amine and the virulence factors phenotypic tested.

Significance: Obtained results indicated the relevance of identifying virulence-related genes in bacteriocinogenic strains, demanding care in their usage as starter cultures or biopreservatives due to the possibility of horizontal gene transfer to other bacteria in food systems. Acknowledgments: CAPES, CNPq and FAPEMIG.

P1-161 Genomic Comparison of Extended Spectrum β-Lactamase-producing Bacteria Isolated from Beef Cattle Grazing on Pasture

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Introduction: According to the Centers for Disease Control and Prevention, there are at least 2,049,224 illnesses and 23,000 deaths attributed to antibiotic resistance each year. In 2003, the Food and Agriculture Organization and World Health Organization released a statement indicating that the major transmission pathway of resistant bacteria and their genes from the agricultural environment to humans is through consumption of food-producing animals.

Purpose: The purpose of this study was to compare antibiotic resistance genes, specifically extended-spectrum β-lactamase (ESBL) genes, among bacteria isolated from commercial beef farms in North Florida.

Methods: Fecal samples were collected from two separate cohorts of multi-breed beef calf populations derived of Brahman and Angus cattle in 2013 and 2014. Neither set of calves had been previously exposed to antibiotics. Fecal samples were serially diluted and plated on MacConkey Agar containing 4 µg/ml cefotaxime to isolate ESBL-producing bacteria. Whole genome sequencing (WGS) of 31 isolated cefotaxime resistant bacterial isolates from cattle was performed using the Illumina MiSeq. De novo genome assembly was performed using Patric and whole genomes were annotated using RAST.

Results: WGS revealed that the major bacterial species represented by resistant bacteria isolated from farms was *Escherichia coli* (60%) followed by *Shigella flexneri* (20%). The majority of isolates from 2013 were found to contain the $_{bla}$CTX-M-1 gene while 2014 isolates were found contain $_{bla}$CTX-M-32 genes. In addition, all isolates from 2014 were found to be multi-drug resistant compared to 11.8% of isolates from 2013.

Significance: The results of this study demonstrate the high levels of antibiotic resistant organisms shed by cattle not previously exposed to antibiotics. This study also shows the evolution of antibiotic resistance in bacterial populations in food-producing animals over time.

P1-162 *Campylobacter* MLST Subtypes and Antimicrobial Susceptibility of Broiler Cecal Isolates: A Two-year Study from 143 Commercial Flocks

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Introduction: *Campylobacter* spp. are recognized as important agents of human foodborne gastroenteritis. To monitor trends in food safety and public health, antimicrobial susceptibility testing of *Campylobacter* derived from poultry products and infected patients has become common practice in both regulatory food safety and public health programs.

Purpose: The objective of this study was to assess genetic diversity and antimicrobial susceptibility of *Campylobacter jejuni* and *coli* recovered from broiler ceca at slaughter.

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Methods: The ceca from a broiler were collected from the evisceration line in a commercial processing plant, once or twice weekly for two years. *Campylobacter* were cultured from the cecal contents and analyzed by whole genome sequencing, multi-locus sequence typing (MLST) and antimicrobial susceptibility testing.

Results: Seventy-two of 143 birds examined were positive for *Campylobacter*. A total of 24 sequence-types (ST) were identified (Simpson's Index of Diversity [SID] 0.93) belonging to ten *Campylobacter* MSLT clonal complexes (SID 0.68). Two MLST clonal complexes, ST-353 (53.2%) and ST-828 (17.7%), accounted for the majority of *Campylobacter* isolates recovered. Compared to all other clonal complexes, a significantly higher percentage (P<0.05) of *C. coli* isolates belonging to ST-828 complex were resistant to azithromycin, clindamycin, erythromycin, gentamicin and telithromycin. No significant differences in antimicrobial resistance among clonal complexes were observed for ciprofloxacin, nalidixic acid, or tetracycline. No isolates were resistant to florfenicol.

Significance: Despite high genetic diversity among *Campylobacter* recovered from broiler ceca during this two-year study, antimicrobial resistance to aminoglycosides (gentamicin), macrolides (azithromycin and erythromycin) and lincosamides (clindamycin) was significantly linked to one MLST clonal complex (ST-828).

P1-163 Proteomic and Molecular Study to Identify the Inactivation Mechanisms of a Norovirus Surrogate by Cold Plasma Exposure

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❖ ***Developing Scientist Competitor***

Introduction: Non-thermal decontamination of fresh-eaten foods and food-contact surfaces from foodborne viruses, particularly human norovirus (NoV), is of great interest to food industry personnel. Recently, we reported complete inactivation of feline calicivirus (FCV), a surrogate of human NoV, by 15 second exposure in water to Ar+1%O_2 cold atmospheric pressure gaseous plasma (CAGP).

Purpose: In the present study, we studied the mechanism of virucidal activity of CAGP to understand how viruses are being affected by cold plasma.

Methods: Cultural, proteomic, molecular, and morphologic comparisons of CAGP-inactivated FCV and non-exposed virus were undertaken.

Results: The reactive oxygen and nitrogen species of plasma oxidized specific amino acids (i.e., histidine, methionine, and tryptophan) located in the shell (S) and protrusion (P) domains, the dimeric interface regions, and movable- flexible hinge region linked between S and P domains of the major capsid protein (VP1) of the virus. This led to: 1) loss of the structural integrity and damage to viral capsid in the exposed virions, 2) hindrance in viral attachment to the functional receptor (fJAM-A) of the host cells, and 3) cleavage in the viral RNA once it becomes uncoated because of the damage to the viral capsid. The former effect is not the key role in virus inactivation as the infectivity of the virus is lost by the capsid damage even if the RNA is left intact after plasma exposure.

Significance: The high similarity between the capsid structures of NoV and FCV and the high histidine, methionine, and tryptophan- content of human NoV capsid protein indicate that similar mechanisms may apply to CAGP's effect on NoV. This understanding of how the virus is being affected by plasma and what plasma species are most effective against the virus led us to design a new cold plasma unit, which is more efficient against foodborne viruses.

P1-164 A 2D-Hollow-Air-Based Cold Plasma Generation Unit for Inactivation of a Human Norovirus Surrogate on Food Contact Surface

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❖ ***Developing Scientist Competitor***

Introduction: Non-thermal decontamination of food contact surfaces from foodborne viruses, particularly human norovirus (NoV), is important in food service industry. We have found that a 15 second exposure to Ar+1%O_2 cold atmospheric pressure plasma jet was able to completely inactivate feline calicivirus (FCV), a surrogate of human NoV. Unfortunately, this plasma jet is capable of point exposure only and cannot be used on an industrial scale for decontaminating food contact surfaces.

Purpose: In the present study we evaluated and optimized the use, for the first time, of an atmospheric pressure two dimensional hollow-air-based high voltage cold plasma generation unit for FCV decontamination from food-grade stainless steel (grade 304) surface.

Methods: The effects of dry and wet plasma exposure, plasma generation power, exposure distance, air flow rate, and exposure time on the reduction of FCV titer (projected as log $TCID_{50}$/100 µl of the elution buffer) were studied.

Results: No significant (P<0.05) inactivation of FCV was observed in dry exposure while > 3 log reduction was attained when the surface was wetted by spraying water on the surface prior to plasma exposure. The highest virus inactivation (>3 log reduction in FCV titer) was observed at a 10 W plasma generation power, 3 mm exposure distance between the stainless steel surface and the hollow surface of plasma generation unit, 10 slm air flow rate, and 4 min exposure time.

Significance: The results showed that this 2D- hollow air-based plasma generation unit is more advantageous than the plasma jet unit since it is suitable for treating areas of surfaces plus it generates plasma from air with no need for using expensive pure gases. The results show that this novel plasma unit is promising in industrial application for decontaminating food contact surfaces from foodborne viruses.

P1-165 Elucidation of Molecular Mechanisms of Foodborne Pathogen Inactivation by Cold Plasma through RNA-Seq Analysis

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Introduction: As an emerging technology, cold atmospheric plasma offers a dry, non-thermal, rapid decontamination process with minimal damage to food products, therefore overcoming many of the limitations of current food decontamination methods.

Purpose: Although the bactericidal effects of cold plasma are well documented, the molecular mechanisms and modes of action underlining such effects are not well understood. The purpose of this study was to elucidate the molecular response of *Salmonella* Enteritidis to cold plasma treatment through transcriptomic analysis by RNA-Seq.

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Methods: Culture suspensions of *Salmonella* Enteritidis were spot inoculated (100 µl in 20-25 spots) onto 10 sterile cover slips (10^8 cells/cover slip, 10^6 -10^7 cells/spot) and treated with cold plasma for 2 min at 1 cm from actuators. Three treated and untreated (control) samples (10 cover slips per sample) were washed by vortexing in sterile peptone and the resulting wash fluid was used for total RNA isolation, cell enumeration, and evaluation of bacterial inactivation. rRNA-depleted libraries of isolated RNA were sequenced by Illumina HiSeq and transcriptomic differences between control and treated samples were evaluated using the web-based Activesite Comparative Expression Viewer (Cofactor Genomics).

Results: Among 375 differentially expressed genes (treated and control) with fold changes greater than 2.00 ($P < 0.05$), 101 were moderately up-regulated (fold changes between 2.10 to 5.19) after plasma treatment. Approximately 50% of the up-regulated genes were associated with known *Salmonella* responses to macrophage infection and constituents of the *Salmonella* pathogenicity island 2 (SPI2), indicating possible oxidative stress responses similar to those experienced in the macrophage environment. A majority of genes down-regulated after plasma treatment were associated with nutrient uptake and desiccation stress, indicating a possible shift in cellular response to plasma treatment from desiccation stress (experienced by the control samples) to oxidative stress (experienced by the treated samples).

Significance: These results confirm the key role of oxidative stress in the inactivation of bacterial pathogens by cold plasma treatment. Cold plasma actuator designs can thus be optimized to maximize reactive oxygen and nitrogen species production for effective food decontamination applications.

P1-166 Effects of a Nanoscale Plasma Coating on Virulence Gene Expression in Pathogenic Biofilms

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Introduction: In our previous studies, trimethylsilane (TMS) and TMS+O_2 (1:4) plasma nanocoatings were found to reduce the numbers of *Listeria monocytogenes* in biofilms by 99%. On the surface of TMS and TMS+O_2wafers, an increase in carbon, silicon and oxygen elements was detected and distorted cells were found, indicating deleterious effects of the coatings on cells.

Purpose: The objective of this study was to investigate if the nanocoatings affect virulence gene expression in *L. monocytogenes* and *Escherichia coli* O157:H7.

Methods: *L. monocytogenes* and *E. coli* O157:H7 were allowed to form biofilms on stainless steel (SS) wafers for 48 h. RNA extraction and reverse transcription were conducted on cells released from the biofilms. The expression of the *hly*A, *rpo*B, *inl*A, *inl*B and *act*A genes in *L. monocytogenes*, and *stx*1, *stx*2, *eae*A and *arc*A in *E. coli* O157:H7 was analyzed. Agar pour plating, and laser confocal and scanning electron microscopies were performed to investigate the mechanism of action of the nanocoatings on these pathogens.

Results: On TMS and TMS+O_2 SS, *L. monocytogenes* numbers dropped from 10^7 to 10^5 CFU/wafer, distorted cells were found but no significant reduction in the expression of *hly*A and *rpoB* was detected compared to the uncoated group. The TMS treatment showed a 0.5 log reduction in *E. coli* O157:H7 with some distorted cells but no significant difference in the expression of *stx*1, *stx*2 and *eae*A genes was found.

Significance: With the change in surface elements, the surface attractive forces may increase vastly enough to distort and kill adjacent cells without affecting the expression of virulence genes. The results imply that TMS and TMS+O_2 coatings can reduce the formation of biofilms without leading to resistance to this type of coating.

P1-167 Predatory *Halobacteriovorax*: A Natural Alternative to Antibiotics in Food Safety

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Introduction: The routine use of antibiotics in animal production and disease treatment has led to antibiotic resistance against numerous bacterial pathogens. These bacteria spread into the global food web to infect products and consumers. In addition, food contamination by unsanitary handling or processing contributes to foodborne illnesses worldwide.

Purpose: Alternative "green" technologies are needed to reduce pathogens, including antibiotic-resistant pathogens, in foods. One potential treatment involves the use of predatory bacteria of the genus *Halobacteriovorax*, which are gram-negative, motile, marine organisms which attack, infect, and kill other gram-negative bacteria.

Methods: We isolated from seawater *Halobacteriovorax* against several *Vibrio parahaemolyticus* strains, and against *E. coli* O157:H7, and *Salmonella enterica* serovar Typhimurium DT104 using modified plaque assays. Assays were performed on lawns of different pathogens to determine the ability of *Halobacteriovorax* to infect various host strains. Oysters and seawater were also challenged with a streptomycin-resistant strain of *V. parahaemolyticus* and their levels were monitored as *Halobacteriovorax counts* in natural seawater increased.

Results: Host specificity was narrow for *Halobacteriovorax* strains originally isolated in *V. parahaemolyticus*, but was broader for strains isolated in either *E. coli* or *Salmonella* Typhimurium. *Halobacteriovorax* significantly reduced ($P<0.05$) the levels of *V. parahaemolyticus* in seawater and oysters, indicating a potential use for *Halobacteriovorax* treatment in aquaculture and depuration settings.

Significance: *Halobacteriovorax* may be a practical substitute for antibiotics in reducing fish and shellfish pathogens in aquaculture, while isolates against *E. coli* and *Salmonella* spp. have the potential to reduce pathogens on the surface of meats, fruits and vegetables. *Halobacteriovorax* treatment by soaking or dipping represents a green technology which could easily and effectively reduce pathogens in foods and on their surfaces.

P1-168 Combination of Vinegar Powder and Reduced Pressure Levels in Extending Shelf Life of High-pressure Processed Raw, Ground Beef and Turkey

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Introduction: High Pressure Processing (HPP) at 87,000 psi for 3 min has been used to reduce spoilage microorganisms in food. At these high pressures, some changes in raw food products may be observed.

Purpose: This study was performed to determine the effect of using a combination of vinegar powder and reduced pressure levels in extending shelf life of raw, ground turkey and beef.

Methods: Reduced pressure levels (60,000, 65,000, 70,000 psi for 3 min) and buffered vinegar powder (0.15%, 0.25%, 0.5%) were evaluated. Whole muscle was ground with added vinegar treatment, vacuum packaged (day 0) and then high-pressure processed (day 3). Control samples received HPP and no vinegar treatment. Total aerobic count was evaluated for 57 days by plating 25 g samples (n=2) using APC Petrifilm method.

Results: End of shelf life is typically considered to be 6 log CFU/g total plate count. Vinegar showed a benefit when combined with reduced levels HPP; bacterial counts remained low throughout the shelf-life. Results indicated vinegar (0.25%) and HPP (60000 psi for 3 min) in ground turkey can extend its shelf life up to 60 days; by day 57 bacterial counts increased to 4.52 log CFU/g. Ground beef samples with vinegar (0.15%) combined with 60,000 psi showed 3.66 log CFU/g total aerobic count at day 57. Control ground turkey samples subjected to 60,000, 65,000, and 70,000 psi reached

over in 6 log CFU/g in 28, 48 and 51 days, respectively. Control ground beef samples subjected to 60,000 and 65,000 psi reached end of shelf life in 28 and 50 days, respectively, while at day 57, samples subjected to 70,000 psi remained at 4.67 log CFU/g.

Significance: Vinegar powder at low levels when combined with HPP can help reduce the pressure levels required to extend shelf life of ground beef and turkey products for up to 60 days.

P1-169 Preventing Pathogen Outgrowth in High-pressure Processed, Ready-to-Eat Meat and Poultry Products Using a Secondary Inhibitor

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Introduction: Ready-to-eat (RTE) meat and poultry products are produced using High Pressure Processing (HPP) technology at 87,000 psi for 3 min to inactivate pathogens and reduce spoilage microorganisms. However, when product is opened for slicing, re-contamination can occur with *Listeria monocytogenes* (LM) from deli-slicers, surfaces and counters where the product is handled.

Purpose: The objective of this study was to determine the effect of using buffered vinegar powder at low levels (0.25%) as a secondary inhibitor in high-pressure processed RTE products to prevent outgrowth of LM introduced during slicing after HPP packages are opened.

Methods: Turkey, beef and ham were formulated with vinegar at 0.25% and subjected to 87,000 psi for 3 min; product packages were opened, sliced and surface inoculated with a five-strain cocktail of LM (3 log CFU/g), heat sealed and stored at 39±1°F.Pathogen outgrowth was evaluated (n=3) for 14 days using modified oxford medium (MOX). Control samples received HPP and no vinegar treatment.

Results: Results indicate that pathogen outgrowth (LM, ≥2 log CFU/g) occurs in absence of a secondary inhibitor after high-pressure processed product packages are opened and sliced. In the presence of vinegar, in beef and ham, only 1.81 and 1.03 log CFU/g increase was seen in 14 days. In turkey however, a 2 log CFU/g increase was seen after day 11 and an increase of 2.41 log CFU/g was observed at day 14. Turkey and beef control samples (without vinegar) showed ≥2 log CFU/g increase in LM in 7 days while ham control showed the same increase at day 11.

Significance: Introducing vinegar powder as a secondary inhibitor in RTE meat and poultry formulations can prevent outgrowth of LM in high-pressure processed products once the original package is opened and product is sliced.

P1-170 Efficacy of Dry Buffered Vinegar and Organic Acid Blends for Controlling Mold Spoilage in Semi-Moist Pet Treats

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Introduction: Sales of natural pet treats have significantly increased in recent years. Semi-moist pet treats are typically 20 to 30% moisture with 0.60 to 0.88 water activity and are at risk of fungal spoilage. Potassium sorbate with phosphoric acid is an effective preservative however does not meet the definition of natural for use in pet food applications, limiting the appeal.

Purpose: Evaluate the preservation and palatability effects of buffered vinegar combined with citric acid and/or succinic acid in semi-moist pet treats.

Methods: Agar dilution assays were used to determine Minimum Inhibitory Concentrations (MIC) of buffered vinegar-acid combinations against *Penicillium chrysogenum* and *Eurotium rubrum.* Additionally, semi-moist treats were manufactured with 0.6% of buffered vinegar with citric acid in Trial A and 1.0 % of both buffered vinegar acid combinations in Trial B. Cultured whey and potassium sorbate were also evaluated. Efficacy was determined by triplicate inoculation with *E. rubrum* spores at 10^5, 10^4 and 10^3 CFU/ml. Un-inoculated treats were retained for shelf-life analysis. The number of days until visible mycelial growth occurred was recorded. The impact of treatments on palatability was assessed in two pal tests with 20 dogs over two days. Significant differences in intake ratio were determined.

Results: In agar tests, the MIC of all combinations was found to be 0.6% for *Penicillium* and 1.0% for *Eurotium*. *Eurotium*-inoculated treats were preserved for at up to 30 days in trial A and 90 days in trial B. Untreated and cultured whey treats spoiled in an average of 5 days. Buffered vinegar-acid combinations continue to suppress spoilage in real-time shelf-life treat studies (5 to 7 months), comparable to potassium sorbate. Dog palatability results indicated a significant preference for buffered vinegar treats ($P<0.05$).

Significance: This work demonstrates the potential for buffered vinegar-acid blends to prevent mold spoilage in natural semi-moist pet treats without negative impact on palatability.

P1-171 Virucidal Efficacy of Chemical Disinfectants against Human Norovirus on Food Contact Surface

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Introduction: In recent years, environmental surfaces have frequently been associated with the foodborne transmission of enteric viruses, such as human norovirus. Therefore, several studies have focused on developing methods to inactivate foodborne viruses thus preventing outbreaks of foodborne illnesses.

Purpose: This study was conducted to evaluate the virucidal efficacies of chemical disinfectants against human norovirus GII.4 (NoV GII.4) on various food contact surfaces using immunomagnetic separation (IMS) technique and real time RT-qPCR.

Methods: The NoV GII.4 monoclonal antibody conjugated magnetic beads captured intact viral particles of NoV GII.4 to allow for subsequent quantitative real-time RT-qPCR after disinfection treatments on various surfaces. Viral suspensions of approximately 5.89 log genomic copies were inoculated on glass, stainless steel, polyvinyl chloride(PVC), rubber, wood, and ceramic tile. We evaluated the optimum elution buffer among the various elution buffers and then 0.25 M glycine-0.14 M NaCl (pH 9.5) buffer was decided to use for eluting the NoV from surfaces. The virucidal efficacy of the ethanol, sodium hypochlorite, and quaternary ammonium compounds was evaluated using a modified European CEN EN 1276 method (Dilution-Neutralization Method) based on quantitative suspension testing.

Results: For all kinds of tested surfaces, ethanol (50 to 70%), Sodium hypochlorite (200 to 2,000 ppm), and quaternary compounds(1,000 to 2,000 ppm) were investigated, and the log reduction values among the tested disinfectants were below <1 log except for 2,000 ppm sodium hypochlorite (between 1.04±0.07 to 1.63±0.24 log reductions).

Significance: These results illustrated that the necessity of developing suitable strategy for inactivation the NoV with disinfectants on environmental surfaces. In addition, we suggest that IMS technique could be diversely applied to investigate efficacies of chemical disinfectants against NoV.

P1-172 Efficacy of Oxidizing Disinfectants at Inactivating Murine Norovirus on Ready-to-Eat Foods

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Introduction: Noroviruses are the leading cause of foodborne illness, and ready-to-eat foods are frequent vehicles of transmission. Studies of the disinfection of fruits and vegetables are becoming numerous. It has been shown that strong oxidizing agents are more effective than other chemical disinfectants for inactivating enteric viruses.

Purpose: The aim of this study was to evaluate the efficacy of oxidizing disinfectants at inactivating noroviruses: (1) in suspension and, (2) on fruits and vegetables.

Methods: Sodium hypochlorite, chloride dioxide and peracetic acid were used against murine norovirus in suspension and spiked on strawberries, blueberries and lettuce. Artificial feces were also tested for their potential protective effect against disinfectants. The viruses were recovered after each treatment and the viral reduction (log PFU/ml) was determined by plaque assays.

Results: Solutions of peracetic acid (85 ppm) and chlorine dioxide (20 ppm) reduced the infectivity of the virus in suspension by at least 3-log units after 1 min, while sodium hypochlorite at 50 ppm produced a 2-log reduction. On the surface of blueberries, strawberries and lettuce, chlorine dioxide was less effective than peracetic acid and sodium hypochlorite, which reduced viral titers by approximately 4 logs. A surprising increase in the efficacy of sodium hypochlorite on surfaces fouled with artificial feces was noted.

Significance: These results will help targeting the active ingredients in the formulation of disinfectants for washing fruits and vegetables to reduce the incidence of these pathogens.

P1-173 Inactivation of GI.6 and GII.4 Human Norovirus by Silver Dihydrogen Citrate

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Introduction: Human norovirus is the leading cause of viral gastroenteritis worldwide and can remain infectious on surfaces for weeks. Additionally, the virus is resistant to numerous sanitizers and disinfectants. Therefore, there is a need for the development of improved methods for inactivating human norovirus.

Purpose: The purpose of this study was to investigate the efficacy of a silver dihydrogen citrate (SDC)-containing disinfectant against human norovirus strains GI.6 and GII.4.

Methods: PURE Hard Surface disinfectant (Pure Bioscience, El Cajon, CA) was evaluated using virucidal suspension assays and stainless steel carrier tests according to ASTM International standards. A blend of silver (0.003%) and citric acid (4.846%) in the form of SDC serves as the active ingredients in this product. Inactivation of human norovirus was quantified using RT-qPCR with a prior RNase treatment. Additionally, the impact of SDC against the viral capsid was assessed by transmission electron microscopy, SDS-PAGE/Western blot analysis, and a histo-blood group antigen (HBGA) receptor-binding assay.

Results: Suspension assays showed a 4.0 log reduction in both GI.6 and GII.4 RNA copy number within 5 min exposure. When carriers were used, 30 min of exposure reduced GI.6 and GII.4 RNA concentration by 2.0 and 3.0 log, respectively, indicating human norovirus inoculated onto surfaces was more resistant to SDC than in suspension. Incorporating a soil load into the sample matrix significantly reduced SDC efficacy, which was completely lost for surface-inoculated samples, even after 30 min exposure. Exposure to SDC (30 min) resulted in VLP deformation and aggregation, a 50% reduction in VP1 major capsid protein band intensity, and an 80% reduction in HBGA receptor binding ability.

Significance: These results suggest SDC to work almost exclusively on the capsid of human norovirus. Taken together, the results suggest SDC to be an effective disinfectant for human norovirus when used on pre-cleaned surfaces.

P1-174 In Vitro Characterization of Antilisterial Activity by Bacteriophage Endolysin PlyP100

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Introduction: The Gram-positive pathogen *Listeria monocytogenes* is highly problematic in the manufacture and processing of ready-to-eat foods due to its environmental persistence and its ability to grow under refrigerated storage. Special care must be taken to prevent listerial contamination during the production of fresh cheeses, as their delicate flavor and texture are sensitive to disruption by many of the antimicrobial processes and additives commonly used for other foods. Bacteriophage-derived cell wall hydrolytic enzymes, known as endolysins, comprise one possible intervention and may not suffer from the high strain-specificity of their parent phages.

Purpose: To address limitations of current antilisterial processes for fresh cheeses, by assessing antimicrobial efficacy of a *Listeria*-specific endolysin in vitro.

Methods: The *L. monocytogenes* phage P100 endolysin, PlyP100, was recombinantly expressed in *Escherichia coli* BL21(DE3), purified, and applied to listerial cultures suspended in phosphate buffer at a range of pH levels, temperatures, and salt-concentrations. Turbidity reduction assays were conducted over 30 min periods with optical densities of samples monitored via microplate spectrophotometer.

Results: Turbidity reduction assays confirm that PlyP100 exhibits optimal cell destruction at pH 8, mesophilic temperatures, and 100-250 mM NaCl. All listerial strains subjected to PlyP100, including 18 strains of *L. monocytogenes* and 5 strains of related species, were susceptible to enzymatic degradation, while all other gram-positive organisms tested (*n*=20) were insensitive except for *Bacillus subtilis*, *Clostridium difficile*, *Brevibacterium linens*, and *Lactobacillus plantarum*. Furthermore, we confirmed that sensitivity to PlyP100 is dependent upon the growth phase of target cultures.

Significance: This study demonstrates the potential of bacteriophage endolysins in targeting foodborne pathogens and highlights factors to consider for their study and application in foods, including target specificity and environmental tolerance.

P1-175 Probiotic Potential of Lactic Acid Bacteria Isolated from Fermented Taro Skins

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Introduction: *Salmonella* and *Listeria* have caused a large number of foodborne illnesses and great economic loss in food and animal production. Piglets recover faster from gastrointestinal illness when fed fermented taro skins. Our previous work shows that lactic acid bacteria (LAB) dominate the microbial population of the taro skins.

Purpose: This study aimed to determine the physiological characteristics and potential health benefits of the isolated LAB.

Methods: Cooked taro skins were obtained from four poi manufacturers in Hawaii. After natural fermentation, LAB isolates were obtained on MRS agar and identified by 16S rDNA gene sequencing. Representative LAB strains were tested for their acid and bile tolerance and antimicrobial activity. We also explored the production of bacteriocins by the LAB isolates.

Results: A total of 159 isolates belonging to 11 different LAB species were obtained, of which 40% were *Leuconostoc mesenteroides*. Four LAB isolates exhibited high tolerance to low pH and bile. *Salmonella* Typhimurium and *Listeria monocytogenes* died in their culture supernatants within 2 h and 8 h, respectively. Proteinase K treatment reduced the inhibition zone of certain supernatants against *L. monocytogenes*. After neutralization, only the supernatant of isolate #137 *Lactococcus lactis* still retained the bactericidal activity against *L. monocytogenes.* In PCR test, two bacteriocin structural genes were amplified by primers NisZ and Bac147 from its genomic DNA. #137 is the first *Lactococcus lactis* strain reported to carry these two bacteriocin genes simultaneously. The anti-*Listeria* bacteriocins produced by the strain were heat-stable but sensitive to proteinase K and pronase E treatments.

Significance: Both organic acids and bacteriocins may account for the antimicrobial activity of the LAB isolates. These isolates could potentially be used as probiotics to fight bacterial infections and confer other health benefits in humans and animals. Bacteriocins produced by the isolates would offer a promising biocontrol tool for the inhibition of pathogenic bacteria in food.

P1-176 Antimicrobial Activity of Essential Oil Emulsions and Possible Synergistic Effect on Foodborne Pathogens

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❖ ***Developing Scientist Competitor***

Introduction: Industrialization has led to major improvements in food quality and safety, yet food borne infections remain an important public health concern. Essential oils are well-known as natural antimicrobials and a good source of combination therapy. However, their application is limited due to high minimum inhibitory concentration (MIC) and insolubility in water. Application of emulsions may offer a solution.

Purpose: The study was aimed to evaluate antimicrobial and synergistic potential of essential oil emulsions against *E. coli* (ATCC 25922), *E. coli* (ATCC 700927), *L. monocytogenes* (ATCC 19115), *L. innocua* (ATCC 33090) and *S. Typhimurium* (ATCC 19585).

Methods: Cinnamon, oregano, clove, thyme, rosemary, sage, bergamot, nutmeg, lemon grass, bay and also t-cinnamaldehyde, euganol and carvacrol emulsions were formulated using ultrasonication and tween 80 as an emulsifier. Antimicrobial efficacy and synergistic potential of t-cinnamaldehyde emulsion was evaluated against the test strains using broth-dilution test and checkerboard assay. To determine the antimicrobial efficacy of the selected essential oils and their combinations in a food model, chicken samples were treated and inoculated. They were stored at 4°C and analyzed on day 0, day1, day3 and day6 for bacterial growth.

Results: Cinnamon showed the highest antimicrobial efficacy against all test organisms at 312.5 ppm. Oregano had the second highest efficacy at 625 ppm, while the other oils did not exhibit high antimicrobial activities. The only synergism observed was between cinnamon and oregano against *E. coli* (ATCC700927) and L. innocua (ATCC 33090) and also between cinnamon and clove towards *L. innocua* (ATCC 33090). All other combinations were additive or indifferent in nature to the test organisms. Cinnamon in comparison to control showed Log reduction of *E. coli* (ATCC25922), *E. coli* (ATCC 700927), *L. monocytogenes* (ATCC 19115), *L. innocua* (ATCC 33090) and *S.Typhimurium* (ATCC19585) by 2.885, 3.39, 3.275, 4.29 and 3.06 in the chicken samples. While oregano reduced *E. coli* (ATCC25922) and *E. coli* (ATCC700927) by 3.21 and 3.53 Log.

Significance: Antimicrobial emulsion of select essential oils and their combinations have the potential to be used as alternative antimicrobial agents for enhancing food safety.

P1-177 *Lactobacillus plantarum* B391 Bacteriocin *ex-situ* Studies Using Fresh Cheese and Pork Meat

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Introduction: Naturally occurring molecules able to inhibit the growth of food pathogens, as bacteriocins, are of great interest to the food industry due to their potential role in food safety

Purpose: A bacteriocin produced by *Lactobacillus plantarum* B391, has been characterized and its use has been tested for the prevention of *Listeria monocytogenes* growth in food products.

Methods: The effect on the reduction of an initial load of *Listeria monocytogenes* spiked on the food materials tested was determined by plating on ALOA agar. Bacteriocin B391 activity was determined using the spot-on-lawn method and successive dilutions.

Results: The bacteriocin B391, a small peptide of 6KDa, showed to be very stable in a range of pH between 3.95 and 8.09. Its thermal stability is very high but pH dependent, being thermolabile at alkaline pH. In the present work, the activity of the purified bacteriocin was tested in fresh cheese and also in pork meat. In fresh cheese, when using an initial inoculum (10^5 CFU/ml) of *Listeria monocytogenes* in the presence of bacteriocin B391, more than 1 log of reduction of the initial population was obtained in a period of 2 months at 4ºC. The bacteriocin B391 was also used to obtain a coated plastic packaging film (PA/EVOH/PE). This film was tested in pork previously contaminated with *Listeria monocytogenes*, and it was possible to observe a reduction of 1 log after 24 h at 4ºC

Significance: The bacteriocin B391 characteristics, and *ex situ* tests incorporating or using the bacteriocin directly on food systems demonstrated its potential use in industry, namely to extend shelf life and promote food safety in particular of products where *Listeria monocytogenes* is able to grow.

P1-178 Preparation of Buttermilk Peptide Extract That Has Antimicrobial Activity against Avian Pathogens

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Introduction: Antibiotics are commonly used in poultry production as growth promoters. This practice has been called into question given the growing problem of antibiotic resistance. Antimicrobial peptides may hold promise as effective alternatives to antibiotics. To this end, large amounts of active preparations of antimicrobial peptides need to be produced, at low cost, from inexpensive food substrates using large-scale commercially viable processes.

Purpose: The objective was to develop a rapid and efficient method for preparing buttermilk cationic peptide extract and to test the antimicrobial activity against specific enteric avian pathogens.

Methods: Buttermilk was digested with pepsin. The ultrafiltered peptide extract (<10 kDa) was fractionated according to the charges through high-capacity cation-exchange adsorptive membrane. Using the microdilution technique on microplates, antimicrobial activity was assessed against

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four avian pathogens: *Salmonella enterica* var. Enteritidis, *Salmonella enterica* var. Typhimurium, two strains of *Escherichia coli* and again *Staphylococcus aureus*.

Results: The yield of buttermilk cationic peptide extract accounted for 6.5 ± 1.8% (*n*=3) of the total buttermilk extract. The buttermilk cationic peptide extract showed bactericidal activity against the selected avian strains, with bacterial losses of 1.7 log CFU/ml (*Salmonella* Typhimurium) to 3 log CFU/ml (*E. coli* O78:H80) at < 5 mg/ml; the effectiveness of cationic peptide extract varied with the strains. Using the same method of preparation, cationic peptide extract from lactoferrin hydrolysate showed bactericidal activity at 0.6 mg/ml (*E. coli* O78:H80) and 2.5 mg/ml (*Salmonella* Typhimurium).

Significance: These results demonstrate that membrane adsorption chromatography is an effective way to prepare cationic peptide extracts from buttermilk that have antimicrobial activity against avian pathogens.

P1-179 Antimicrobial Activity of Gums on the Growth and Antibiotic Susceptibility of Foodborne Pathogens

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Introduction: Gums are a group of complex polysaccharides obtained from plant, seaweeds, bacteria, and animal sources. In addition to enhancing viscosity and the growth of probiotics in food, some gums are found to have bacteriostatic properties.

Purpose: The objective of this study was to investigate the synergistic effect of gums on the growth and antibiotic susceptibility of foodborne pathogens.

Methods: Batches of 200 ml sterilized tryptic soy broth containing 0.5% gums were inoculated at 0.05% and plated on tryptic soy agar to determine initial bacterial counts and then final counts after incubation at 37°C for 16 h. Antibiotic activity was determined by disc diffusion on tryptic soy agar containing 0.5% gums.

Results: Amongst antibiotics tested, treatment with carrageenan-maltodextrin (52.00±2.00 mm) and pectin (51.00±1.00 mm) resulted in a two-fold increase in susceptibility of *Salmonella enterica* to imipenem 10 µg, compared to the control (25.33±1.00 mm). Treatment with locust bean and agar also led to a two-fold increase in susceptibility of *Salmonella enterica* to cefixime and xanthan also increased susceptibility of *Escherichia coli* O157:H7 to doripenem. Compared to control, addition of locust bean, pectin and xanthan led to the highest growth inhibition of *Salmonella enterica*, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Listeria monocytogenes,* respectively. Bacterial survival decreased in the presence of all gums with pectin showing a 2.68±0.10 log CFU ml^{-1} reduction during refrigerated storage.

Significance: These findings suggest that carrageenan, pectin, locust bean and agar could potentially be used in combination with antimicrobial agents to improve their efficacy in the treatment of *Salmonella enterica* and *Escherichia coli* O157:H7 infections resulting in early recovery. Additionally, these gums could be used together with other ingredients to preserve food, thus, reducing dependence on chemicals and promoting food safety.

P1-180 Metal Detectable Brush Bristles – Myth or Miracle

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Introduction: Foreign body contamination of foods can be a safety or quality issue, or both. Regardless, if a food is contaminated by a foreign body, the repercussions for the food business can be expensive and damaging. Consequently, the food industry constantly seeks ways to minimize the risk of foreign bodies in food, including the use of metal detection. One source of foreign body contamination is food industry cleaning brushware, where the bristles can snap, be cut, or detach from the brush head and enter the food product. Recently, brushes with 'metal detectable' bristles have been marketed to the food industry as a way of detecting foreign bodies from this source, but do they work?

Purpose: To investigate the durability, functionality and detectability of metal detectable brush bristles

Methods: Durability – metal detectable and plastic bristles were investigated with regard to their break strength and elasticity using a Zwicki 5kN (Zwick Roell). Functionality - the ability of metal detectable bristled brushware to clean a surface of a wet and a dry food soil, was compared with that of a standard plastic bristled brush, using a robotic cleaning rig (Vikan). Metal detectability - metal detectable bristles were investigated with regard to their detectability using a commercial metal detector (Mettler Toledo), with and without the presence of a wet and a dry food product.

Results: Metal detectable bristled products were equally as effective at cleaning as standard drilled and stapled products. PBT bristles were 68% stronger and more than twice as elastic as metal detectable bristles. Metal detectable bristles were not detectable in the presence of a food simulant.

Significance: Metal detectable bristled brushware offers no advantage with regard to cleaning efficacy and is unlikely to minimize the risk of bristle contamination of food, in fact, it may increase it.

P1-181 Efficacy of Commercial Citrus-based and Chemical Preservatives against Survival of *Campylobacter jejuni* In Vitro and in a Food Model

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Introduction: *Campylobacter* is associated with foodborne gastroenteritis in humans, and chicken plays a very important role for microbial dissemination. *C. jejuni/coli* have emerged over last decades as a significant pathogen of humans causing 400-500 million cases of campylobacteriosis each year worldwide. **Methods** to control contamination of these bacteria in foods are not completely successful.

Purpose: To determine the efficacy of commercial, citrus-based and synthetic, preservatives and their mixtures, against survival of *C. jejuni* in vitro and in a food model.

Methods: Minimal Bactericidal Concentration (MBC) of Trisodium Phosphate (TSP), Citrosan and Citrol was determined against two strains of *C. jejuni*. The synergistic effect of preservative mixtures was determined using the checkerboard method. The most effective combination was evaluated in chicken wings using the Fisher method. Sensory analysis of this mixture was conducted by a semi-trained panel using the duo-trio method.

Results: The MBC against *C. jejuni* of TSP, Citrosan and Citrol were 0.5 ± 0.04%, 0.05 ± 0.0006% and 0.0006 ± 0.0001%, respectively. Combinations lower than individual MBC inhibited microbes, among them TSP:Citrosan at 0.4:0.03 %, TSP:Citrol at 0.3:0.0003% and Citrosan:Citrol 0.4:0.0005%. When mixtures were analyzed in chicken wings, the combinations 2:0.3:0.05% TSP:Citrosan:Citrol and 0.3:0.05% Citrosan:Citrol, both showed a reduction of at least 4 log of *C. jejuni* by 48 h. Sensory analysis showed no significant difference among the mixtures when compared with the chicken wings without preservatives.

Significance: Mixtures of TSP, Citrosan and Citrol at concentrations lower than those suggested for individual use in the food industry, significantly reduced survival of *C. jejuni* in chicken wings.

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P1-182 Synthesis and Antimicrobial Study of Nanoporous Metal-Organic Frameworks (MOFs) Loaded with Thymol

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Introduction: With growing demands for food safety and public health, there has been large interest in developing food preservatives, especially using natural antimicrobials to improve the food safety and quality. Thymol is one of the essential oils found in oregano with antifungal, insecticidal, antimicrobial, and antioxidant abilities, and it can be potentially ideal as a food preservative if its major drawbacks can be addressed, including undesirable aroma and low solubility in water.

Purpose: To solve this limitation, loading and encapsulation are the main approaches to form micelles and nanoparticles, as it promotes better dispersing of thymol in water as well as maximizing the surface area of bacteria and thymol interaction.

Methods: In this study, for the first time, we have demonstrated a simple surfactant-free post-synthesis loading process of metal-organic frameworks (MOFs) with thymol. We have synthesized a MOFs (defined as Zn@MOF) from zinc nitrate hexahydrate and 2- aminoterephthalic acid (defined as Zn@MOF). Thymol was then loaded into the Zn@MOF pores. The whole process does did not require any surfactants or templates under relative mild conditions (105°C). The structure of porous crystal MOFs was confirmed using scanning electron microscopy (SEM) and X-ray diffraction (XRD).

Results: Thymol was then loaded inside the MOFs with a loading rate of 3.95%. This method produced crystalline Zn@MOF with nanoscale porous cavities loaded with thymol, which were confirmed by multiple characterization methods. The antimicrobial experiment showed promising antimicrobial ability of T-Zn@MOF. Inhibition of *E. coli* O157:H7 was measured both in tryptic soy broth (TSB) medium and on TSA agar. An *E. coli* O157:H7 reduction of 4.4 log CFU/ml was achieved at a thymol to broth ratio of 0.04 g/100 g. An inhibition area of 223.73 mm^2 was observed after 12 hours incubation.

Significance: Although still at the very early stages of development, the T-Zn@MOF that we produced acts as a pioneer in the application of MOFs loading antimicrobial agents in the food.

P1-183 Characterization and Antimicrobial Activity of Polypropylene Films Containing AgSio2, AgZ and Ag-Zn Useful for Returnable Container for Seafood Distribution

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Introduction: Present study reports the returnable antimicrobial effect and physio-chemical characterization of polypropylene (PP) films containing Ag-Sio2, Ag-Z (silver zeolite) and Ag-Zn for application of seafood packaging.

Purpose: The purpose of the present study was to develop the returnable package/container use full during distribution/transportation of seafood.

Methods: Three master-batches containing AgSio2, Ag-Z and Ag-Zn were mixed with PP in different compositions (1%, 3%, 5%, 10%) and extruded to produce plain films. A complete thermal, structural, mechanical and functional characterization of all formulations was carried out. The morphology of the films showed the formation of agglomerates of particles in composites. A decrease in the elongation was obtained for active formulation compared to pure PP Sample. Thermal analyses indicated an increase in thermal stability of the active formulation compositions. The antimicrobial efficiency was determined by applying the test strain gram-negative *Pseudomonas aeruginosa, Shewanella putrefaciens*, *Escherichia coli* and gram-positive *Clostridium, Listeria monocytogenes, Staphylococcus aureus,* as target microorganisms by disk diffusion and, as per the Japanese Industrial Standard Method (JIS Z 2801:2000). After following the standard procedure of washing for returnable plastic, the antimicrobial efficiency again tested for (10%) formulation.

Results: The films showed 50% antimicrobial properties after 5th wash against the tested microorganisms, presenting better activity against the gram negative than gram positive.

Significance: These findings suggest that PP films with AgSio2, Ag-Z and Ag-Zn are promising to provide a significant contribution to the quality and safety of packaged seafood.

P1-184 In Vitro Assessment of the Antimicrobial Activity of Emerging Chemical Disinfectants against Guaiacol-producing *Alicyclobacillus acidoterrestris* Isolated from Orchard Soils

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❖ ***Developing Scientist Competitor***

Introduction: *Alicyclobacillus acidoterrestris* is a thermo-acidophilic, endospore-forming bacterium which causes spoilage of pasteurized fruit juices with the production of guaiacol taints.

Purpose: This study investigate the occurrence of *A. acidoterrestris* in orchard soils in Nigeria and the in vitro efficacy of poly-dimethyl-ammonium-chloride (PDAC) and chlorhexidine disinfectants against the bacterium.

Methods: *A. acidoterrestris* was isolated from soils by heat shock and sample enrichment in acidified yeast-starch-glucose (YSG) broth at 45°C for 5 days. The isolates were identified by 16S rDNA sequencing followed by PCR-RAPD for strain typing. High performance liquid chromatographic analysis was used to detect guaiacol in YSG broth supplemented with vanillic acid and inoculated with the isolates. Subsequently, the cells and endospores of the isolates were treated with the disinfectants (0.5 to 20%) using disk diffusion method. The isolates were then exposed to the disinfectant solutions for 5, 15 and 30-min contact times. The disinfectant-treated cultures were grown in YSG broth and growth was measured at 24 h intervals for 144 h. The effect of the disinfectants on the isolates was viewed using scanning electron microscopy (SEM).

Results: Four *A. acidoterrestris* strains were identified in the orchard soils and PCR-RAPD grouped these isolates into two genotypes. The four isolates produced guaiacol from vanillic acid with guaiacol concentrations ranging from 27 to 35.6 mg/ml. Disk diffusion assay showed that the tests strains were all sensitive to both disinfectants at all concentrations tested. Contact-time assay showed that 5 min exposure to 0.5% PDAC and 5% chlorhexidine disinfectants inhibited the growth of the isolates. Scanning electron micrographs showed alterations in the outer membrane of disinfectant-treated cells relative to that of untreated *A. acidoterrestris* cells, indicating damage caused by the disinfectants to the bacterial cell membrane.

Significance: The two disinfectants showed tremendous potential for use in the inactivation of *A. acidoterrestris* during fruit juice processing however, further in situ studies may be required to ascertain their effectiveness under real-life industrial conditions.

Poster

P1-185 Biomimetic Molecularly Imprinted Polymers: A New Quorum Sensing Capturing Agent to Prevent Bacterial Biofilm Formation

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Introduction: Biofilms are prevalently formed by monospecies and multispecies bacteria in food processing environments. Compared to planktonic cells, biofilms are up to 10-500 times more resistant to antimicrobial agents and disinfectants, thus constituting a threat to public health and food industry. The formation of biofilms is mediated by quorum sensing (QS). QS is a mechanism by which bacteria can assess their population density through the secretion and sensing of autoinducers (AIs) and regulate the expression of certain genes in a cell-density dependent manner. Attenuating QS via sequestration of AIs represents a promising strategy to control biofilm development. However, conventional sequestration agents (*i.e.*, antibodies) are unstable and their large-scale production is difficult to achieve. To address these disadvantages, molecularly imprinted polymers (MIPs), known as "artificial antibodies," that were developed to specifically capture AIs.

Purpose: The overall objective of this study was to develop a MIP and evaluate its inhibitory effect against bacterial biofilm formation.

Methods: *Pseudomonas aeruginosa* was selected as it is a model organism for the study of both biofilms and QS. As one of the major AIs produced by *P. aeruginosa*, N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C_{12}-AHL) was served as the template molecule for the synthesis of MIPs. Binding capacity and selectivity of MIPs towards 3-oxo-C_{12}-AHL was evaluated in equilibrium binding tests in aqueous condition (*i.e.*, 20% acetonitrile). To evaluate the inhibitory effect of MIPs against biofilm formation, biofilms of *P. aeruginosa* and/or *Salmonella enterica* and *Campylobacter jejuni* were incubated with or without the presence of MIPs. Confocal laser scanning microscopy staining assay and crystal violet staining assay were conducted to quantify biofilm formation levels. Statistical significance was determined using Student's *t*-test.

Results: The binding capacity of MIPs was 3.81 mg/g, which was 1.22 times higher than the control group. It indicated highly specificity of MIPs towards 3-oxo-C_{12}-AHL.The results showed that MIPs could significantly ($P<0.05$) prevent biofilm formation at the early and middle stage (*i.e.*, 4 to 12 h).

Significance: This study developed and evaluated a novel strategy to control both monospecies and multispecies bacterial biofilm formation and can be potentially applied to inhibit other QS-regulated bacterial behaviors.

P1-186 A Wash Treatment of "Fit-L" on Cattle for Reduction of Foodborne Pathogens and Its Safety on Eye Evaluation

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Introduction: Although considerable effort was applied to reduce *E. coli* O157:H7 and *Salmonella* on, effective hide treatment for pathogen removal is still needed.

Purpose: To evaluate the efficacy of commercial "Fit-L" product as a wash treatment on cattle for reduction of foodborne pathogens.

Methods: Beef cattle were selected. The right side of cattle was used for "Fit-L" treatment and left side was used for water-only treatment at 45-55 psi for 30 s. The face of 3 cattle was washed with "Fit-L and 3 was washed with water-only. Sponge samples of the hides were collected at before treatment, 5 and 10 min after treatment and enumerated for *Escherichia coli*. An eye-drop containing 0.2% levulinic acid plus 0.02% sodium dodecyl sulfate was applied to all eyes of the cattle for observation of any stimulus symptoms.

Results: The average *E. coli* count before washing was 6.6 log CFU/cm^2. For tap water only washed cattle the average *E. coli* count was 6.0 log CFU/cm^2 at 5 min and 6.1 log CFU/cm^2 at 10 min. Whereas, for "Fit-L"-washed cattle the average *E. coli* count was 2.6 log CFU/cm^2 at 5 min and 2.3 log CFU/cm^2 at 10 min. These data revealed that a simple "Fit-L" wash could reduce *E. coli* population by 3.4 log and 3.8 log on the surface of cattle hide at 5 min and 10 min, respectively; when compared with tap-water wash only. A tap water-only wash reduced *E. coli* by 0.5 log CFU/cm^2 when compared with samples collected before the wash. Following "Fit-L" washing with one more tap water wash did not further reduce *E. coli* on the surface of cattle hides. The application to use it as an eye-drop did not produce any stimulus symptoms on all cattle eyes.

Significance: These results suggested a simple "Fit-L" wash just before cattle entered the slaughter facility will substantially reduce the population of *E. coli* on cattle hides.

P1-187 Efficacy of Sulfuric Acid Sodium Sulfate to Reduce Inoculated Populations of *Salmonella* and *Campylobacter* on Pork Subprimals

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Introduction: *Salmonella* and *Campylobacter* are pathogens commonly associated with foodborne illness. As these pathogens are often found in fresh pork, efforts to reduce or eliminate them is imperative to the pork industry.

Purpose: This study was conducted to determine efficacy of sulfuric acid sodium sulfate (SA) to reduce inoculated populations of *Salmonella* spp. and *Campylobacter* spp. on pork subprimals. Additionally, this study aimed to determine efficacy of SA against inoculated populations of non-pathogenic *Escherichia coli* that could then serve as surrogates for *Salmonella* spp. and *Campylobacter* spp. on pork.

Methods: Vacuum packaged pork subprimals were obtained from a local retailer less than 10 days postmortem. Entire subprimals were cut into uniform sample pieces and assigned to one of the following treatments: 1.0 pH SA, 1.5 pH SA, water or an untreated control. Samples were inoculated to a target level of 6 log CFU/g for *Salmonella* spp. and surrogate *E.* coli, or 5.5 log CFU/g for *Campylobacter* spp., with cocktails prior to treatment. Surviving pathogen and non-pathogenic *E. coli* populations were determined at 5 min post-treatment and 24 h post-treatment.

Results: Application of 1.0 pH SA was the most effective ($P<0.05$) at reducing inoculated populations of both *Salmonella* and *Campylobacter* compared to all other treatments. However, no difference ($P>0.05$) was observed for *Campylobacter* and surrogate bacterial populations determined at 5 min versus populations at 24 h. Additionally, non-pathogenic *E. coli* strains demonstrated a smaller reduction when compared to inoculated *Salmonella* and *Campylobacter* populations and can therefore effectively serve as surrogates for *Salmonella* spp. and *Campylobacter* spp.

Significance: Overall, treatment with SA is an effective method for reducing pathogens on fresh pork and implementation of SA spray in pork processing facilities can help reduce foodborne illnesses associated with fresh pork consumption.

Poster

P1-188 Enhancement in Thermal Inactivation of *Cronobacter sakazakii* by Inclusion of Parabens

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❖ ***Developing Scientist Competitor***

Introduction: *Cronobacter sakazakii*has been linked to fatal invasive infections in infants associated with low-level contamination of powdered infant formula. It is controlled through effective pasteurization of infant formula prior to dehydration. Parabens are a group of p-hydroxybenzoic acid esters, which possess both antioxidant and antimicrobial activity. Past research has established that the relative antimicrobial activity of parabens in food increase with the length of alkyl side chain. In addition, parabens have antimicrobial activity over a broad pH range, i.e. 4-8.

Purpose: The purpose of this study was to evaluate the enhanced thermal inactivation of *Cronobacter sakazakii* by the inclusion of parabens and to ultimately develop mathematical models that describe the relationship between heating temperature, parabens identity, and parabens concentration.

Methods: *C. sakazakii*607 (heat-resistant) was subjected to five parabens (methyl, ethyl, propyl, butyl, heptyl) in various concentrations under three temperatures (52°C, 55°C, 58°C). Thermal inactivation was conducted in a submerged coil apparatus using Brain Heart Infusion as the heating menstruum. Cells were surface plated on tryptic soy agar (TSA) and MacConkey agar (MA). After enumeration, survivor curves were plotted and compared using ANOVA. Primary and secondary models were developed using OriginPro etc.

Results: Parabens produced a significant enhancement of thermal inactivation that was concentration dependent and increased with increasing alkyl chain length. For example, at 58°C butyl-paraben, at concentration of 0 ppm, 31.25 ppm, 62.5 ppm, 125 ppm, resulted in log reductions of 2.5, 4.0, 5.5, >7.0, respectively, within 900 seconds. At a concentration of 125 ppm in conjunction with heating at 58°C, methyl, ethyl, propyl, butyl, and heptyl parabens produced log reductions of 3.0, 3.5, 5.5, 7.0, and >7.0, respectively. The comparison of TSA vs. MA counts indicated 0.5-2.0 log of injury. The three parameters (concentration, alky side chain length, and heating temperature) acted synergistically on thermal inactivation of *C. sakazakii*607, even at temperatures that little impact was observed with the 0 ppm controls.

Significance: These data suggest that pasteurization can be enhanced through synergistic action of mild heat treatments and inclusion of parabens. These data can be used to develop mathematical models that effectively describe how these three process parameters can be applied in the industry.

P1-189 Cultures as a Natural Antimicrobial for Food Biopreservation: Example of *Leuconostoc mesenteroides* Inhibition in Bacon

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Introduction: Recent analyze of US bacon from the market has shown that a significant part of the products reaches during their shelf life a high bacterial load (with mainly undefined lactic acid bacteria). Such uncontrolled microflora may negatively impact shelf life and bring significant economic losses.

Purpose: This study focused on application of bioprotective culture on bacon with two main objectives: (i) growth inhibition of *Leuconostoc mesenteroides* (one of the bacteria mostly involved in the bacon spoilage) to extend shelf life and (ii) clean label, a strong customer trends.

Methods: Six batches of bacon (a control without culture and 5 different starter cultures) were produced using standard US recipe and production process. Several parameters were measured along the product shelf life: (i) the *Leuconostoc mesenteroides* concentration through challenge testing and (ii) the sensory features through color and pH measurement, evaluation of slime formation and smell.

Results: Among the tested cultures, a *Leuconostoc carnosum* strain has shown the best *Leuconostoc mesenteroides* inhibitory effect. After 80 days of vacuum storage at 41°F, the spoilage growth was limited to 2 log units when the *Leuconostoc carnosum* strain was added while in the control batch, the spoilage growth potential was close to 6 log unit. No slime formation, nor off smell was detected in the batch with added culture. No difference in color (red intensity) was measured. Finally a slight pH difference (-0.1 pH Unit) was measured at the end of the shelf life between the treated sample and the control. Additional studies have shown that the *Leuconostoc carnosum* strain used is able to produce a bacteriocin and also to strongly dominate the microflora of meat products. Those two features mainly explain the inhibitory effect of this unique strain.

Significance: These results suggest that the tested *Leuconostoc carnosum* strain significantly improves bacon microbial quality.

P1-190 Growth Inhibition of *Cronobacter sakazakii* in Experimentally Contaminated Powdered Infant Formula by Kefir Supernatant

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❖ ***Developing Scientist Competitor***

Introduction: Kefir is a type of fermented milk containing lactic and acetic acid bacteria and yeast.

Purpose: In this study, we evaluated the antimicrobial activity of kefir supernatant against *Cronobacter sakazakii* in powdered infant formula (PIF).

Methods: Antibacterial activity was assessed by the spot-on-lawn method, agar well diffusion assay, measurement of optical density of culture broths. The antimicrobial activity of kefir supernatant to experimentally contaminated PIF was also investigated by plate count method.

Results: In a spot-on-lawn test, the growth of 20 *C. sakazakii* strains—including 10 clinical and 10 food isolates—was completely inhibited in the presence of kefir supernatant. Significant differences in the diameters of inhibition zones were observed upon treatment with kefir as compared to *Lactobacillus kefiri* and *Candida kefyr* culture supernatants or solutions of lactic and acetic acid and ethyl alcohol in the agar well diffusion test ($P < 0.05$). The addition of 100-µl kefir supernatant to 1 ml of nutrient broth completely inhibited the growth of *C. sakazakii*, as evaluated by spectrophotometry. The antimicrobial activity of kefir supernatant in experimentally contaminated PIF was also tested; we found no viable *C. sakazakii* cells remaining in PIF rehydrated with 30% kefir supernatant solution for 1 h.

Significance: This is the first study demonstrating the antimicrobial effects of kefir against C. sakazakii. Kefir has antimicrobial effects against both clinical and food-isolated C. sakazakii strains and could kill viable C. sakazakii in real PIF samples. In conclusion, the antimicrobial activity of kefir supernatant against *C. sakazakii* could be applied in real food samples.

Poster

P1-191 Synthesis, Characterization and In Vitro Evaluation of Chitosan-Monomethyl Fumaric Acid Conjugate for Antibacterial and Antioxidant Activities

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❖ ***Developing Scientist Competitor***

Introduction: Chitosan is known for its antimicrobial and antioxidant activities around the world. However, the insolubility of chitosan in aqueous solution at pH >6.0 limits some of these potential uses.

Purpose: The present study was carried out to modify chitosan with monomethyl fumaric acid to enhance its solubility, antibacterial and antioxidant activities.

Methods: Conjugation of chitosan with MFA in acidic medium was achieved by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as mediator. Three different kinds of conjugate samples 1, 2 and 3 were synthesized by feeding different concentration of MFA. The resulting materials were characterized by ^{1}H NMR, ^{13}C NMR, High-resolution XRD, and TNBS assay. Solubility, antibacterial and antioxidant activities of the conjugates samples have been studied by solubility test, 96 well-plate assay, and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay, respectively.

Results: The ^{1}H NMR, ^{13}C NMR, HRXRD confirmed the successful synthesis of conjugates samples 1, 2 and 3. The degree of substitution (DS) for each samples ranging from 1.82±0.05% to 7.88±0.04%. Conjugates sample 1, 2 and 3 are readily soluble in water and swelled by dimethyl sulfoxide (DMSO), toluene and dimethylformamide (DMF). The antioxidant activity for all the conjugate samples have been significantly improved ($P<0.05$) compared to the native chitosan. Upon antibacterial activity at pH 4.0, all the conjugate samples showed significant ($P<0.05$) antibacterial activity against gram-positive *Staphylococcus aureus* strains, *Listeria monocytogenes* strains and gram-negative *Escherichia coli* strains and *Salmonella* Enteritidis strains compared to native chitosan.

Significance: MFA-modified chitosan has shown enhanced antioxidant and antibacterial activities along with solubility, and could be used as a novel food preservative and packaging material for long time food safety and security.

P1-192 Cranberry Extracts as Natural Antimicrobials in Foods

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Introduction: Plant-derived products have been known for their antimicrobial properties, which could enhance the food safety and increase the shelf life of foods.

Purpose: The goal of this study was to investigate the efficacy of three cranberry extracts: Extract-A, Extract-B and Extract-C, as inhibitors of pathogenic and spoilage bacteria in vitro and in food models to determine their feasibility as natural antimicrobials for food products.

Methods: Cranberry extracts were tested against *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* cells and *Bacillus cereus, B. licheniformis,* and *Paenibacillus lautus* spores using agar dilution assay at pH 5.0 and 6.0 at 37°C. In food models, 2% milk (pH 6.7) and apple juice (pH 3.4) were used for further evaluation at 12.5 and 25°C over 48-h period.

Results: In vitro, Extract-C effectively prevented growth of vegetative pathogens at 0.05-1.0%, with *L. monocytogenes* being most susceptible. Extract-A and B at 1.5% were effective at pH 5.0 against *L. monocytogenes*. For spores, Extract-C was also the most effective product and activity was greater at lower pH. *B. cereus* spores were the most susceptible to Extract-C at 0.1% at pH 5.0. Extract-A at 2% inhibited all spores tested at pH 5.0. In milk, 1% Extract-C could slow the growth of *E. coli* and *Salmonella* over 48 h at 12.5°C, while none of bacterial spores were affected. Extract-C reduced *E. coli*, *Salmonella* and *L. monocytogenes* to undetectable level (~6.0-log reduction) within 24 h in apple juice at 12.5 and 25°C. About 0.7-log reduction of *B. cereus* was achieved with 0.25% Extract-C in apple juice at 48 h.

Significance: Overall, Extract-C was the most effective antimicrobial, with the highest total phenolics, compared to Extract-A and B, showing the potential for use in low-fat, low-protein foods (e.g., beverages and fruit products) and may be effective at higher pHs than most other commonly used traditional antimicrobials.

P1-193 Expression of Antiviral Cytokines against Murine Norovirus by the Treatment of Flavonoids

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Introduction: Human noroviruses (hNoVs) are considered as significant causes of viral gastroenteritis worldwide. As culture system of hNoVs has been absent, antiviral drugs or vaccines were not developed. Recently, there are several studies about flavonoids that have potential as novel antiviral agents. However, their mechanisms were not investigated extensively.

Purpose: The purpose of this study was to investigate the expression of antiviral cytokines and interferon-stimulated genes (ISGs) against human norovirus surrogates, murine norovirus (MNV) by the treatment of flavonoids.

Methods: According to the previous study, six antiviral flavonoids were selected as candidates. In order to examine the expression of antiviral cytokines, 150 μM of EGCG, 100 μM of ECG, fisetin, and quercetin, 50 μM of fisetin and daidzein were pretreated on RAW264.7 cells for 24 h and challenged with MNV. The cells and cell supernatant were harvested in 48 h. The mRNA expression of interferon-α (IFN-α), interferon- λ (IFN-λ), Interleukin-1 (IL-1), Interleukin-6 (IL-6), Tumor necrosis factor-α (TNF-α), zinc finger antiviral protein shorter isoform (ZAPS), Mx, 2′-5′-oligo (A) synthetase (OAS), and inducible nitric oxide synthases (iNOS) were analyzed with measured using quantitative real-time RT-PCR. For the cell supernatant, IFN-α, IFN-β, IL-1, IL-6, TNF-α protein were assayed with ELISA kit.

Results: In mRNA level, IFN-α and TNF-α were increased in of MNV-infected group treated with 50 μM of fisetin or daidzein or 100 μM of quercetin than group infected with MNV alone. IFN-λ and Mx was only highly expressed in MNV-infected group treated with 50 μM of daidzein and 100 μM of quercetin, respectively. Especially, IFN-α protein level showed the significant induction in MNV-infected group treated with 100 μM of fisetin or quercetin.

Significance: IFN-α, IFN-λ, TNF-α, Mx contribute to inhibit the replication of MNV on RAW264.7 cells pretreated with 50 μM of fisetin and daidzein and 100 μM of quercetin and fisetin.

P1-194 Inhibitory Effect of Herbal Extracts against Hepatitis A Virus

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Introduction: Hepatitis A virus (HAV) is positive-sense and single-stranded RNA virus of the *Picornaviridae* family and cause acute liver infection and gastrointestinal infections through the fecal-oral route. Although antimicrobial activity of herbal extracts was studied against bacterial pathogens, it is rare to investigate anti-HAV property of food components.

Purpose: The aim of this study was to examine the inhibitory effect of 16 edible herbal extracts against HAV.

Methods: *S. flavescens, C. officinalis, V. vinifera, P. lactiflora, A. thumbergii, E. ulmoides, E. sessiliflorus, P. multiflorus, C. sativum, G. biloba, A. fistulosum, A. annua, A. sativum, T. japonica, A. pilosa, A. japonica* were used to prepare herbal extract in this study. Antiviral activity, cytotoxicity, and antioxidant activity was measured on FRhK-4 Cells for HAV at 1, 10, and 50 μg/ml concentration of plant extracts. The pre-, co-, and post-treatment of plant extracts was investigated on HAV-infected cells.

Results: Viral inhibition was observed by co-treatment than pre- and post-treatment. The effect of *A. japonica* of 50 μg/ml showed the significant reduction of 3.36 ± 0.17 log PFU/ml of HAV without cytotoxicity. The titer of HAV significantly reduced to 1.84 ± 0.24, 1.77 ± 0.35, and 1.74 ± 0.10 log PFU/ml on FRhK-4 Cells co-treated with 50 μg/ml of *A. pilosa, T. japonica*, and *A. sativum*, respectively. Also, 50 μg/ml of *E. sessiliflorus, P. multiflorus, C. sativum, G. biloba, A. fistulosum, and A. annua* showed the reduction about 1 log PFU/mL against HAV.

Significance: The herbal extracts of *A. japonica*, *A. pilosa, T. japonica*, and *A. sativum* could have potential to control HAV without cytotoxicity.

P1-195 Geraniol-loaded Polymeric Nanoparticles Reduce Pathogen Loads on Fresh Cantaloupe, Spinach, and Tomato Surfaces

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❖ ***Developing Scientist Competitor***

Introduction: The use of nano-encapsulated antimicrobials for fresh produce decontamination represents an innovative technology for preventing pathogen transfer to consumers.

Purpose: The objective of this research was to evaluate geraniol-loaded polymeric nanoparticles (GPNs) to reduce *Escherichia coli* O157:H7 and *Salmonella* Typhimurium inoculated onto produce surfaces.

Methods: For each sample, unwashed cantaloupe, spinach, and tomato surfaces were portioned into three 10 cm^2 pieces. Samples were spotted with a cocktail of rifampicin-resistant *S. enterica* Typhimurium LT2 and *E. coli* O157:H7 ATCC 700728 and held to allow inoculum attachment. Treatments of GPNs (0.5 wt.% delivered concentration, prepared by flash nano-precipitation), unencapsulated geraniol (0.5 wt.%), and 200 ppm HOCl (pH 7.0) were applied via immersion for 2 min. Untreated inoculated samples were prepared. Following immediate enumeration of pathogens, samples were packed and stored aerobically at 5°C. Changes in pathogen numbers were quantified by selective/differential enumeration after 3, 5, 7, and 10 days' storage. On day 5 of incubation, a set of identically prepared and treated samples was transferred to 15°C to simulate post-packing temperature abuse; changes in pathogen numbers were assayed at 7 and 10 days' total storage. Pathogen numbers were analyzed for differences in numbers as a function of treatment application and time of storage (N=3; P<0.05).

Results: Immediately following inoculation and treatment, pathogen numbers were 1.5-6.1 log CFU/cm^2 across treatments for all produce. At day 10, pathogen numbers were 1.0-5.8 log CFU/cm^2 across treatments for cantaloupes and tomatoes and 1.8-3.0 log CFU/cm^2 for chlorine-treated and untreated spinach, yet numbers for GPN- and unencapsulated geraniol-treated spinach were below detection (0.5 log CFU/cm^2). During 15°C storage, pathogen loads increased across treatments for cantaloupes and decreased for GPN-treated tomatoes and spinach.

Significance: Results suggest GPNs can be utilized for the post-harvest decontamination of produce from contaminating enteric pathogens.

P1-196 Antimicrobial Effects of Hydroxytyrosol and Oleuropein Extracted from *Olea europaea* on Major Enteric Bacterial Pathogens

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❖ ***Developing Scientist Competitor***

Introduction: Due to the growing multi-drug resistance in foodborne bacterial pathogens, alternative antimicrobials are essential. The olive, *Olea europaea* is rich in bio-active phenolic compounds like Oleuropein (OP) and hydroxytyrosol (HT) which might be potential in antioxidant, antimicrobial, and anti-inflammatory activities.

Purpose: The purpose of this study was to evaluate the antimicrobial property of HT and OP against growth and virulent gene expression of major foodborne pathogens.

Methods: The minimum bactericidal/inhibitory concentration (MBC/MIC) of HT and OP on enterohemorrhagic *E. coli* EDL933 (EHEC) and *Salmonella* Typhimurium LT2 (ST) were determined by broth micro-dilution method. The inhibitory effects of HT and OP against EHEC and ST were investigated in liquid culture condition. Several invasion-related virulence genes of ST and EHEC under stress of HT and OP were also evaluated by qPCR.

Results: The MBC/MIC of HT on EHEC and ST was found as 0.1% (w/v)/0.075% and 0.075%/0.05%, and it was 1.9%/1.7% and 1.1%/0.8% for OP, both of which were bactericidal. Following a dose-dependent manner, 0.05%, 0.1%, and 0.2% HT reduced 2.8, 3.3, and 4.9 log CFU/ml ST and 2.9, 3.1, and 3.4 log CFU/ml EHEC at 24 hours. Moreover, in mixed-culture with LC, ST and EHEC were excluded quickly by 0.05% and 0.2% HT, respectively, at 24 hours, whereas growth of LC was not affected. 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 by 1.5-6 fold.

Significance: HT in olive might be applied as a strong alternative antimicrobial; OP, both showed antimicrobial effect and in favor of *Lactobacillus* growth, may serve as gut microbiome modulator for prevention of gut foodborne infections.

P1-197 Determination of Aflatoxin Levels in Macadamia Nuts

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Introduction: Aflatoxin contamination in tree nuts, including macadamia nuts, pose a serious health risk to humans, so an accurate and reliable analytical method to determine the concentration of aflatoxin in the samples is required. High-performance liquid chromatography (HPLC) is an attractive method for identification and quantification of aflatoxins because it can provide accurate and precise analytical results. Effectiveness and reduced labor required for analysis.

Purpose: The purpose of this study was to evaluate an HPLC method developed specially for the determination of the aflatoxins in nuts that naturally contaminated with aflatoxin.

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Methods: Ground macadamia nuts were extracted using a 60:40 methanol: water solution and then diluted 1:2 in phosphate buffered saline solution (PBS). Clean up was performed using immune-affinity columns and samples were analyzed by HPLC using KOBRA cell derivatization.

Results: Immuno-affinity column effectiveness was gauged by spiking a non-detect samples with an aflatoxin standard and comparing the resulting value with the spiked value. Recovery values for our spiked samples ranged from 85% - 108%.

Significance: The developed HPLC method is an effective means of determining aflatoxin concentration in macadamia nuts reducing matrix interference and providing high accuracy and precision.

P1-198 Evaluation of Toxicity of Chitosan Nanoparticles with Intestinal Epithelial Cell and *Caenorhabditis elegans*

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❖ ***Developing Scientist Competitor***

Introduction: A natural antimicrobial agent, chitosan nanoparticles (CN), has previously demonstrated broad-spectrum antimicrobial activity. CN have many potential applications in the food industry and offers an alternative to traditional antibiotics. However, it has not been determined whether CN may cause adverse side effects in humans and animals.

Purpose: The purpose of this study was to evaluate the toxicity of CN toward intestinal epithelial cells, Caco-2 cells, and an animal model, *C. elegans*.

Methods: Four types of CN were prepared by cross-linking of chitosan. Two cross-linkers, sodium sulfate (SS) and tripolyphosphate (TPP), and two types of chitosan, low and high molecular weight, were used to generate CN. Caco-2 cells were treated with 0.1, 0.2 or 0.4% CN for 24 h. The morphological change of the cells was checked immediately following treatment. Cellular membrane damage was assessed by lactate dehydrogenase (LDH) assay. In addition, the four types of CN from 0.1 to 0.4% were administered to *C. elegans* for survival assay. The viability of *C. elegans* was monitored every two days for 22 d.

Results: No change in cell morphology or cell viability was observed in Caco-2 cells treated with CN compared to the control. However, we observed a mild level of toxicity in the animal model. In the *C. elegans* survival assay, CN generated with TPP showed lower toxicity compared to the ones generated with SS. The molecular weight of chitosan did not affect the toxicity.

Significance: This data demonstrates that CN are not toxic toward intestinal epithelial cells, but are toxic in the animal model. These results will help with future development of animal models for the risk assessment of newly developed agents.

P1-199 Toxicity Assessment of Secondary Metabolites Extract from *Clitocybe nuda* as Natural Food Antimicrobial

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❖ ***Developing Scientist Competitor***

Introduction: *Clitocybe nuda* is an edible mushroom which shows promise to be developed as novel natural food antimicrobials due to various antimicrobial components found in its secondary metabolites extract in recent years. This antimicrobial of secondary metabolites extract has several advantages such as naturally occurred compounds, against broad spectrum of foodborne pathogens, high water solubility, wide pH range, good thermal stability and low cost. However, currently there is no toxicity information available about this secondary metabolites extract from *C. nuda*.

Purpose: To assess the toxicity effects of the secondary metabolites extract from *C. nuda* using in vitro toxicity testing methods.

Methods: The cytotoxic effect of the secondary metabolites extract was investigated with cytotoxicity assay using both animal and human cell lines. The acute oral LD_{50} values were also estimated based on an established prediction model. Besides, genotoxic effects of this secondary metabolites extract were evaluated with two standard in vitro genotoxicity assays: bacteria reverse mutation assay and cytokinesis-blocked micronucleus cytome assay. Moreover, the potential influences of heat treatment to the secondary metabolites extract toxicity were also investigated.

Results: The estimated acute oral LD_{50} values of this secondary metabolites extract are higher than the threshold value of 2,000 mg/kg body weight, which can be classified as non-toxic according to the state-of-the-art recommendation. Also, there are no significant differences (P<0.05) between this secondary metabolites extract and controls for all of the genotoxic biomarkers examined in both bacteria and human hepatoma cells. Results from this study indicate that the secondary metabolites extract from *C. nuda* is not genotoxic and might also be properly classified as non-acute-oral-toxic.

Significance: Results from this study can help to prove the safety of the secondary metabolites extract from *C. nuda* to be added into foods as a novel natural antimicrobial.

P1-200 Composting: A Biological Process for Aflatoxin Decontamination in Agricultural Environment

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Introduction: In developing countries, there is a high occurrence of aflatoxin contamination in peanuts due to climate conditions and handling practices. Contaminated peanut wastes and shells are often used as soil amendments and mulching materials, which re-introduces aflatoxins and aflatoxin-producing molds into subsequent farming seasons.

Purpose: This research evaluated whether composting can be used to control aflatoxin contamination in agricultural environment by using peanut meal with a high level of aflatoxin contamination as a model matrix.

Methods: The peanut meal was uniformly mixed with deionized water. The samples were inoculated with either one of the 3 commercial starters alone or in combination with a commercial accelerator. The control was peanut meal without the starters or accelerator. Samples were incubated at 40°C in a water bath for 6 weeks. Compost temperature, pH and ammonia concentration were documented twice a day during the process. Aflatoxin B1, B2, G1 and G2 were quantified at the end of each week using high performance liquid chromatography. Two replicate experiments were performed and data obtained were analyzed statistically.

Results: Results showed that the composting resulted in a significant reduction in the amount of aflatoxin B1, B2, G1 and G2 in peanut meal during the 6 week experiment. The average amounts B1, B2, G1 and G2 decreased from 195.40 to 80.92 ppb, 22.17 to 10.05 ppb, 2.89 to 0.09 ppb, and 1.20 to 0.17 ppb, respectively. The reduction range of B1, B2, G1 and G2 were found to be 72.16-154.95 ppb, 7.42-17.56 ppb, 1.18- 6.94 ppb, and 0.00-2.10 ppb, respectively. Mean compost temperature, pH and ammonia contents ranged from 21.5.0°C to 48.30°C, 5.60 to 8.25 units, and 0 to ≥ 500 ppm, respectively, at different stages of the composting process.

Significance: The research demonstrates that composting is effective means to decontaminate aflatoxin. The process has the potential to reduce the level of aflatoxin contamination in agricultural environment.

P1-201 Incidence and Mycotoxigenic Potentials of Fungi Isolated from Some Traditionally Fermented Foods in Nigeria

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Introduction: Fungi and their toxins have drawn global attention due to the significant threat they pose to food safety.

Purpose: This study assessed the occurrence of fungi in some traditionally fermented foods (TFF) and evaluated the toxigenic potential of the fungi isolated from the TFF.

Methods: Cluster sampling procedure was used to obtain 18 samples each of fermented maize gruel (FMG), fermented locust beans [FLB] and fermented melon (FM) from different markets in Southwest Nigeria. The fungal load of the samples was determined and macroscopic, microscopic and molecular identification of isolated fungi species was done. The potential of the fungi isolates to produce aflatoxins, deoxynivalenol, fumonisin, ochratoxin and zearalenone was determined using thin-layer chromatography.

Results: The mean fungal load of the FMG, FLB and FM samples were 8.0 x 10^3 CFU/ml, 9.4 x 10^3 CFU/g and 7.9 x10^5 CFU/g, respectively. A total of 164 fungal isolate from nine genera; Aspergillus, *Penicillium, Fusarium, Rhizopus, Saccharomyces, Pichia, Mucor, Candida and Rhodotorula* were identified from the samples. The most dominant fungi isolates in FM samples were *A. flavus*, *A. parasiticus*, *F. verticillioides* and *P. rugolosum* while *S. cerevisiae* was identified from all the FMG samples. A total of 99 potentially toxigenic isolates of *Aspergillus* (56), *Penicillium* (24) and *Fusarium* (9) species were recovered from the samples. All positive extracts for aflatoxin G_1 and zearalenone had spots with retardation factors ranging from 39 - 41mm and 80 - 85 mm, respectively. Of all the *Aspergillus* species from the FLB isolates (23), only 65% were positive for aflatoxin B_1 by giving a light blue fluorescence under ultraviolet light while 14% of the fungi extracts from the FM samples were positive for fumonisin B_1 from *F. verticillioides*.

Significance: The TFF are unsafe for consumption due to high fungi load and the consequent presence of mycotoxigenic fungi.

P1-202 Investigation of the Mycotoxin Contamination in Enzyme Foods by Using Multi-mycotoxin Analysis with HPLC-MS/MS

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Introduction: Mycotoxins are natural toxic contaminants produced by toxigenic fungi and they show a wide range of adverse effects including carcinogenic and immunosuppressive effects. Enzyme foods are processed products which are produced by fermentation of plant materials with edible microorganisms for the purpose of enzyme enrichment. Most of the enzyme foods and the pseudo-enzyme foods consist of cereals, which is vulnerable to mycotoxin contamination.

Purpose: The base data for safety evaluation on mycotoxins in enzyme foods and pseudo-enzyme foods is needed. The purpose of this study was to investigate the level of mycotoxin contamination in the products.

Methods: The samples were 50 enzyme food products and 53 pseudo-enzyme food products marketed in Korea. They were loaded on a Myco 6in1™ immuno-affinity column for extraction and purification. For mycotoxin analysis, we optimized a simultaneous analysis method using HPLC-MS/MS.

Results: The number of samples contaminated with deoxynivalenol (LOD = 0.020 ppm) and zearalenone (LOD = 20 ppb) were 40 (0.021~0.541 ppm) and 11 (22~98 ppb), respectively. Four samples were contaminated with aflatoxin B_1 (3.4~3.9 ppb; LOD = 0.3 ppb) and ochratoxin A were also detected in 4 samples (0.4~1.2 ppb; LOD = 0.3 ppb). Two samples were contaminated with fumonisins (0.04, 0.05 ppm; LOD = 0.025 ppm).

Significance: The contamination level of mycotoxins in the samples of enzyme food and pseudo-enzyme food did not exceed the regulation level of Korea. However considering the harmful effects of mycotoxins to human even at a low levels, it is essential to consistently monitor the mycotoxin levels in the products.

P1-203 Traditional Post-harvest Management Practices of Maize among Smallholder Farmers in the Western Highlands of Guatemala and Its Implications in Mycotoxin Contamination

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Introduction: Mycotoxicosis, both acute and chronic, is a reality for developing countries where a combination of traditions and low income leads to a grain-based diet. Maize, one of Guatemala's staple grains, is prone to mycotoxin contamination when improper practices during planting, harvesting, drying and storage take place.

Purpose: To perform an assessment on maize handling practices as well as an evaluation of mycotoxin exposure in the Huehuetenango department in Guatemala.

Methods: A survey was conducted among farmers (n=280) from Todos Santos and Chiantla, townships of Huehuetenango. Additionally, aflatoxin and fumonisin were measured in samples from 25 households in the region employing a lateral-quantifiable ELISA. Range of detection was 0-100 ppb (LOD=3.6 ppb, LOQ=5.0 ppb) for aflatoxin and 0-5 ppm (LOD=0.3 ppm, LOQ=0.4 ppm) for fumonisin. Due to lack of normality in the mycotoxin data, a Recursive Partitioning Model (RPM) was used for analysis.

Results: The survey revealed that most (88%) farmers dry the maize by a direct sunlight exposure, primarily as cobs. During the storage time, 61% of farmers perform grain quality checks once a week. Moreover, 65% perform pest control during storage but only as a corrective measure. Forty-nine percent of farmers indicated mishandling of grain moisture as the main cause of maize loss, leading to insect infestation and fungal growth. RPM revealed that farmers who buy maize are at higher risk of fumonisin contamination; while aflatoxin was present for both producers and buyers. Afla-

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toxin was found in all farmers' maize while fumonisin was in 52%. Aflatoxin levels were influenced by the relative humidity (>74.5%) of the storage location. Alarmingly, the mean of all detectable cases of mycotoxin contamination were above the Provisional Maximum Tolerable Daily Intake (PMTDI).

Significance: Farmers of the highlands of Guatemala need to improve their agricultural practices in order to have maize and maize-based safe products.

P1-204 Simultaneous Determination of Multi-Mycotoxins in Cereal Grains by LC-MS/MS

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Introduction: Aflatoxins (B_1, B_2, G_1, and G_2), ochratoxin A, fumonisins (B_1 and B_2), nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol/15-acetyldeoxynivalenol, zearalenone, T-2 toxin, and HT-2 toxin are major groups of mycotoxins found in agricultural crops. Since these mycotoxins can occur simultaneously in cereal grains, there is a need to determine the levels of the multi-mycotoxins in grains.

Purpose: The purpose was to establish a sensitive and reliable analytical method for simultaneous determination of 13 mycotoxins in cereal grains by liquid chromatography-mass spectrometry (LC-MS/MS) and to monitor the levels of mycotoxins in samples collected from retail markets in South Korea by using the established analytical method.

Methods: We evaluated the linearity, sensitivity, specificity, and accuracy of the analytical method for determination of mycotoxins in cereal grains by LC-MS/MS after improved toxin extraction and purification using Myco6in1$^+$ immunoaffinity columns. The method used to determine the levels of multi-mycotoxins in 500 cereal grains (100 each of brown rice, millet, sorghum, maize, and mixed cereal samples).

Results: The mycotoxin calibration curves had strong coefficient of determination ($R^2 > 0.99$). The mycotoxin recovery rates were 100 – 119% (brown rice), 93.6 – 112% (millet), 88.2 – 102% (sorghum), 91 – 101% (maize), or 80.1 – 113% (mixed cereal) with % RSD of 0.57 – 7.89%, 0.25 – 9.17%, 0 – 1.97%, or 0.12 – 2.92%, 0.06 – 17.52%, respectively, at 8 – 326 ng/g of the spiked levels. The limits of quantification of the method for the mycotoxins were in the range of 0.6 - 6.0 µg/kg for all cereal grains. The incidence of the mycotoxins in samples collected from South Korean retail markets were 35%, 40%, 49%, 35%, and 34% in brown rice, millet, sorghum, maize, and mixed cereal, respectively.

Significance: The established method was suitable for assessing the incidence of multi-mycotoxins in cereal grains sampled from South Korean retail markets.

P1-205 Biodegradation of Ochratoxin A by *Aspergillus tubingensis* Isolated from *Meju*

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Introduction: Ochratoxin A (OTA), a mycotoxin, contaminates food commodities and poses a serious threat to public health worldwide. OTA is one of the most potent carcinogenic (IARC group 2B) mycotoxins. Microbiological methods are known to be an appropriate approach for biodegradation of OTA which is occasionally found in fermented soybean products.

Purpose: For microbiological reduction of OTA in fermented soybean products, fungi that have OTA-biodegradation activity were isolated from *meju*, a traditional starter for Korean soybean fermented products. The OTA-biodegradation activity of 7 fungi and their crude enzymes was investigated.

Methods: One hundred thirty fungal species were isolated from *meju* and their OTA-biodegradation activity was evaluated. Biodegradation of OTA was analyzed by thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC). Seven fungal isolates that show OTA-biodegradation activity were identified by sequencing β-tubulin gene and the Internal Transcribed Spacer (ITS) region of rDNA.

Results: Seven *Aspergilli* were selected as OTA-biodegradable abilities and identified as *A. tubingensis* M013, M024, M036, M038, M069 and M074. They degraded more than 90% of OTA in 14 days in Soytone-Czapek broth and converted OTA to a much less toxic metabolite, ochratoxin α. Their crude enzymes degraded more than 85% and 75% of OTA at pH 5 and pH 7, respectively, for 24 h.

Significance: Our data suggests that *A. tubingensis* M013, M024, M036, M038, M069 and M074 isolated from *meju* samples and their OTA-biodegradable enzymes have potential for practical applications to reduce OTA levels in soybean fermented food and feed processing.

P1-206 Degradation and Detoxification of AFB_1 by Two *Pseudomonas* Species Isolated from a South African Gold Mine Aquifer

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Introduction: Food commodities suffer severe colonization by filamentous and toxigenic fungal strains, accompanied by the production of mycotoxins. Aflatoxins (AFs), principally aflatoxin B_1 (AFB_1) represent the most toxic mycotoxin group along the food chain and presents one of the most daunting challenges to combat in food safety.

Purpose: Considering the adverse effects associated with this toxin, there is need for strategies to reduce or at best, eliminate their occurrence in food commodities. This study thus investigated the AFB_1 biodegradation ability of liquid cultures and cell lysates of *Pseudomonas anguilliseptica* and *Pseudomonas fluorescens* and the cytotoxicity of the degraded products thereof.

Methods: Liquid cultures and cell lysates (disrupted in the presence or absence of protease inhibitors to obtain lysates) were respectively incubated with 2.5 µg/ml AFB_1 for 3, 6, 12, 24 and 48 h. Aflatoxin B_1 degradation was subsequently monitored on high performance liquid chromatography (HPLC), while cytotoxicity of the bio-transformed extracts were investigated against human lymphocytes using the MTT assay.

Results: After 48 h of incubation, liquid cultures of *P. anguilliseptica* demonstrated significantly (P<0.05) higher % AFB_1 degradation of 64.9% as compared to *P. fluorescens* of 59.9%. Conversely, in 6 h of incubation, 83.5 and 79.1% AFB_1 degradation was observed for the protease inhibited lysates of *P. anguilliseptica and P. fluorescens*, respectively, while in 12 h, a 100% AFB_1 degradation was recorded. The uninhibited lysates however recorded a significantly (P<0.05) lower percentage AFB_1 degradation. Results obtained from the cytotoxicity studies showed that the AFB_1 bio-transformed products exhibited a significantly (P 0.05) lower cytotoxic effect when compared to the parent AFB_1.

Significance: This study demonstrated the efficacy of crude bacterial lysates to degrade and detoxify AFB_1. The potential of these lysates in decontaminating AFB_1 could be an important bio-control measure with a huge potential for industrial applications.

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P2-01 Behavior of Different *Salmonella* spp. Strains in Black Pepper (*Piper nigrum*), Oregano (*Origanum vulgare*) and White Pepper (*Piper nigrum*)

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Introduction: It has been established that *Salmonella* spp. can survive for long periods of time in foods with low water activities such as peanut butter and spices. In fact, some studies have reported *Salmonella* spp. prevalence in spices as high as 40% and different *Salmonella* spp. outbreaks have been traced back to this kind of products.

Purpose: The objective of the study is to analyze some factors affecting the behavior of *Salmonella* spp. in black pepper, white pepper and oregano.

Methods: Samples were inoculated with low (2 log CFU/g) and high (9 log CFU/g) levels of a *Salmonella* spp. cocktail (5 strains) using a spray method and drying inside a biosafety cabinet (12 h). High population samples were stored at 25 and 35°C for 2 months to determine survivability; these samples were also used to evaluate the thermal resistance of *Salmonella* spp. at 65°C. Low population samples were incubated at 25°C to evaluate growth after increasing the water activity of the product (0.991) with peptone water. *Salmonella* spp. populations were recovered on tryptic soy agar.

Results: *Salmonella* spp. was able to survive up to 61 days (both temperatures) in black and white pepper although the population decreased during storage. A shorter survivability (41 days) was observed in oregano at 25°C. $D_{65°C}$ values of 5.7 ± 0.8 and 4.28 ± 0.4 min were observed for white and black pepper, respectively. Under high water activity conditions, *Salmonella* spp. population increased more than 4.0 log CFU/g in white pepper stored at 25°C but shorter increases (1.1 log CFU/g) were observed for black pepper.

Significance: These data demonstrate that there is an increased risk for persistence of *Salmonella* spp. in some herbs and spices that are used as raw material for other types of food products.

P2-02 *Salmonella* Survival in Dried Garlic Products

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Introduction: *Salmonella* has been frequently associated with contamination of low moisture foods including spices. However, survival kinetics of *Salmonella* in many dried seasoning products has not been well established. Recent recalls of dehydrated garlic due to potential *Salmonella* contamination point to the need to characterize *Salmonella* survival in such products despite the well-known natural antimicrobial properties of garlic.

Purpose: To characterize and model *Salmonella* survival in dehydrated garlic granules of various sizes under different storage conditions.

Methods: A four serotype cocktail of *Salmonella* was inoculated into both fresh garlic and dehydrate garlic flakes to investigate 1) *Salmonella* survival during thermal dehydration at 75 to 85℃ for 190 min and 2) dried garlic storage at 25 and 35℃ under low and high relative humidity (RH) conditions up to 88 days. Survival kinetics of *Salmonella* was further modeled by inoculating the cocktail into dehydrate garlic granules of different sizes and incubating at different levels of water activity (a_w) for 7 to 22 days.

Results: Over 6-log reduction was observed after dehydration of fresh garlic at 75 to 85℃ for 190 min. During storage, *Salmonella* population (7.63 CFU/g) dropped below the limit of detection (<1.59 CFU/g) within 9 days under both low and high relative humidity at 35℃, but persisted up to 88 days at 25℃ with 3.35 log CFU/g remaining under low RH, and 73 days before dropping below limit of detection under high RH. Weibull modeling of survival kinetics showed that storage under moderate humidity (a_w=0.728) led to the longest decimal reduction time in dehydrate garlic granules of all sizes. Difference in survival kinetics was observed between different granules, which also appeared to have different levels of allicin.

Significance: This study provides quantitative data for risk assessment of *Salmonella* in dehydrated garlic and highlights the potential risk of *Salmonella* persistence during long term storage of dry garlic under certain conditions. Our results also suggest different aw/RH levels may have different effects on *Salmonella* survival during the storage of dried spices with natural antimicrobial activity.

P2-03 Effects of Temperature, Water Activity, and Structure on Thermal Resistance of *Salmonella* in Dates and Date Paste

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✦ ***Undergraduate Student Award Competitor***

Introduction: Various low-moisture products have been implicated in salmonellosis outbreaks and related recalls. However, few studies have addressed *Salmonella* reduction in dried fruits, even though it has been shown to be present and able to survive long periods in such products. Additionally, the effect of structure change (whole fruits vs. fruit paste) on *Salmonella* thermal resistance is not yet well studied.

Purpose: The objective was to quantify the thermal resistance of *Salmonella* on dates and in date paste during heat treatment at different water activities (a_w).

Methods: Date surfaces and pitted dates (later processed into paste) were inoculated with *Salmonella* Enteritidis PT30 and equilibrated in controlled-humidity chambers to 0.25, 0.45, or 0.65 a_w. Samples (~1.7 g) were treated isothermally (in triplicate) in sealed containers in a water bath (70, 75, or 80°C) for defined periods. *Salmonella* survivors were recovered on modified trypticase soy agar, incubated for 48 h, and enumerated. *D*-values were determined from linear regression of the survivor curves for each treatment.

Results: *D*-values decreased (P < 0.05) with increasing temperature (8.5, 2.8, and 1.1 min, respectively) at 0.25 a_w. At 80°C, *D*-values were not affected (P > 0.05) by a_w (1.1, 1.3, and 1.0 min, respectively). *D*-values for *Salmonella* were greater in date paste than on date surfaces (P < 0.05) at 0.45 a_w and 80°C (3.4 and 1.3 min, respectively).

Significance: In most food products, a_w plays a significant role in the thermal resistance of *Salmonella*, but this appeared less true for dates. Other compositional factors may be more important than a_w in this product type. Future studies should continue exploring the effects of these factors to ensure reliable thermal resistance data for process design and validation.

P2-04 Thermal Resistance of *Salmonella enterica* in a High-protein Matrix at Varying Water Activity

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Introduction: Legacy baking processes may need to be validated to satisfy Food Safety Modernization Act (FSMA) requirements. During baking, products typically start out at a high water activity and end at a low water activity. At present, information is lacking on the influence of product composition and dynamic water activity on the thermal resistance of *Salmonella enterica*.

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Purpose: The objectives of this work were to assess the influence of water activity and temperature on thermal resistance of *S. enterica* serovar Agona 447967 in a model high-protein food matrix.

Methods: Plate-harvested cells were used to inoculate a high-protein matrix with a product composition ratio of 43:38:11 (carbohydrate:protein:fat). Varying volumes of buffered peptone water (BPW) diluent were added to adjust water activity (0.50 to 0.98), while holding inoculation level constant. The matrix was hand massaged until visually uniform and then held at ambient conditions (23±2ºC) for one hour to equilibrate. Triplicate samples were packed into aluminum test cells under controlled water activity conditions. Thermal resistance was evaluated under isothermal conditions in an oil bath (54 to 90ºC). Treated samples were serially diluted using BPW, plated on trypticase soy agar with yeast extract, and incubated at 37±2ºC for 48 h. Positive samples were confirmed on xylose lysine deoxycholate agar.

Results: $D_{75°C}$-values were 0.002 to 35.30 min and increased with a reduction in a_w as expected. The z_T-values for a_w 0.501, 0.621, 0.721, 0.803, 0.901, and 0.978 were 9.64, 9.93, 9.89, 10.08, 7.33, 5.22ºC, respectively. Za_w-values exhibited non-linearity and temperature dependence.

Significance: Results indicate that a correlation exists between temperature and water activity and must be accounted for when predicting inactivation of *Salmonella* under dynamic processing conditions.

P2-05 Moisture Equilibration and Product Fabrication Methods Affect Measured Thermal Resistance of *Salmonella* Enteritidis PT30 on/in Whole Almonds, Almond Meal, and Almond Butter

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❖ ***Developing Scientist Competitor***

Introduction: Recent work has suggested that changing the structure of low-moisture food products, with equivalent composition, may affect the thermal resistance of *Salmonella* in/on those products; however, the underlying effects of sample preparation (equilibration and fabrication) have not been systematically evaluated.

Purpose: The objective was to quantify the effect of product equilibration and fabrication on *Salmonella* thermal resistance on/in multiple almond products.

Methods: Whole raw almonds were inoculated with *Salmonella* Enteritidis PT30 (~10^8 CFU/g) and equilibrated (3-10 days) to 0.25, 0.45, or 0.65 a_w (triplicate tests). Inoculated and equilibrated almonds were individually vacuum-packed in plastic bags or fabricated into meal and butter that was loaded into aluminum test cells (~1 g samples). Samples were heated in an isothermal water bath (80°C), pulled at multiple intervals, cooled in an ice bath, diluted in peptone water, and plated on modified trypticase soy agar to enumerate survivors.

Results: Although a_w of almonds appeared to reach equilibrium after 3-5 days (by a_w meter readings), the moisture content (mc) of the meal and butter fabricated from that product were significantly ($P < 0.05$) lower or higher than the almond mc for adsorbing (0.65 a_w) and desorbing (0.25 a_w) conditions, respectively, indicating that full equilibration had not been achieved. However, for 0.25 a_w, after 8-10 days of equilibration, the resulting meal and butter were at the same mc as the almonds, but a_w was higher ($P < 0.05$) for meal (0.29) than butter (0.22) and almonds. Subsequently, the $D_{80°C}$value on whole almonds (19.7 min) was lower ($P < 0.05$) than in meal (50.8 min) and butter (48.3 min).

Significance: Equilibration state impacted a_w changes in fabricated almond products, and product structure may be significantly important when applying inactivation parameters to process validations.

P2-06 Quantifying Reproducibility of *Salmonella* Thermal Resistance through a Multi-laboratory Comparison

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Introduction: Isothermal inactivation studies have been used to quantify *Salmonell*a thermal resistance in various food matrices. Resulting thermal resistance measurements influence research, industry practices, and government food safety guidelines. However, the reproducibility of the methods used in these studies is currently unknown for low-moisture foods.

Purpose: The objective was to quantify the reproducibility of *Salmonella* thermal resistance results in oat flour, via multiple-laboratory comparison.

Methods: Four independently operated laboratories, at the Food and Drug Administration (FDA), Illinois Institute of Technology (IIT), Michigan State University (MSU), and Washington State University (WSU), participated in this study. *Salmonella* Agona lawn cultures were harvested using peptone water and inoculated into three batches of oat flour via liquid addition, which were then equilibrated over 3 days to a water activity of 0.45. Polystyrene bottles containing oat flour samples (100 g) were sent to and subsequently processed by each of the four laboratories using their own isothermal inactivation procedures (80°C). Samples were then serially diluted and plated on trypticase soy agar supplemented with yeast extract. All resultant data were compiled and analyzed collectively.

Results: Average populations of *Salmonella* Agona in the three oat flour samples before thermal treatment were 7.90±0.20, 7.75±0.25, and 7.75±0.29 log CFU/g. Resultant $D_{80°C}$-values were 8.15, 10.70, 15.27, and 18.58 min across the four laboratories. Using a one-way ANCOVA, differences in inactivation rates within laboratories were not significantly different ($P > 0.05$); however, differences in inactivation rates between laboratories were significant ($P < 0.05$).

Significance: Despite the use of identical inoculated matrices, and similar thermal treatment methods, thermal inactivation rates varied significantly among the four laboratories. Lab- or method-dependent artifacts contributing to such differences may limit the ability to utilize results from separate studies, which also suggests that model validation is critical prior to utilizing single-study results for pasteurization validations or regulatory guidance.

P2-07 Evaluation of Thermal Resistance of *Salmonella* Enteritidis PT30 and *Enterococcus faecium* NRRL B-2354 in Wheat Flour and Peanut Butter Using TAC and TDT Cell

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Introduction: *Salmonella* outbreaks in low-moisture foods have created great concerns among consumers, research communities and food processing industry. *Enterococcus faecium* NRRL B-2354 is considered to be an effective surrogate microorganism for *Salmonella* Enteritidis PT30 in validating thermal processing technologies and systems. This research evaluated two factors, namely, food composition and water activity (a_w) on the thermal resistance of *Salmonella* Enteritidis and *E. faecium* in two food models.

Purpose: Using wheat flour and peanut butter as separate model food products inoculated with either *Salmonella* Enteritidis or *E. faecium*: A) Assess inactivation kinetics with a_w=0.45±0.05 at 80 ºC; B) Determine and compare $D_{80°C, 0.45}$ values by using two types of test cells (TDT and TAC cells); C) Explore and find factors (water activity, food types) that influence thermal resistance; D) Identify desorption isotherms (a_w changes with temperature) which influence thermal resistance.

Methods: Organic wheat flour (carbohydrate rich) and peanut butter (oil rich) were inoculated separately with *Salmonella* Enteritidis and *E. faecium* culture (10^{10} CFU/ml), and conditioned to a_w 0.45 with final bacterial populations 10^8 CFU/g after equilibration. Inactivation studies were conducted at 80ºC using TAC and TDT cells in parallel. The TAC cell was able to maintain a constant a_w, while the TDT cell maintained a constant sample moisture content, allowing the elevation of a_w during the heating treatment.

Results: In wheat flour, $D_{80°C, 0.45}$ values (4.25±0.45 min for *Salmonella* Enteritidis, and 5.56±0.49 min for *E. faecium*) by using TDT cells were significantly lower than those (7.28±1.17 min for *Salmonella* Enteritidis, and 9.96±1.06 min for *E. faecium*) by using TAC cells; in peanut butter, $D_{80°C, 0.45}$ values were similar irrespective of the test cells used (TDT: 6.84±0.46 min, TAC: 8.03±0.68 min for *Salmonella* Enteritidis; TDT: 36.12±1.17min, TAC: 37.42±1.68 for *E. faecium*).

Significance: Water activity (a_w) and food type are essential factors influencing the thermal resistance of bacteria. Such factors should considered in further thermal inactivation studies and validation for industrial applications.

P2-08 Thermal Resistance of Osmophilic Fungi in Low-water Activity Confectionery Model Foods

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Introduction: Confectionery products with low water activities typically do not support mold growth, however there are several osmophilic fungi that can cause considerable economic loss by reducing shelf life. Until now, very few studies have been published regarding low water activity (a_w) tolerant molds in food products containing all nutrients essential for mold growth; sugars, carbohydrates, proteins and fats, but not preservatives.

Purpose: The objective of this study was to evaluate the effect of a_w on the thermal tolerance and survival of osmophilic, spoilage fungi *Eurotium repens* and *Neosartorya fischeri*.

Methods: The decimal reduction time (*D*-value) and the temperature needed to cause a 1-log change *D*-value (*z*-value) in a confectionery model food, comprised of evaporated milk and fructose adjusted to 0.70, 0.75 and 0.80 a_w, was determined by creating thermal death time curves with inoculated product at three different temperatures. Experiments were performed in triplicate.

Results: *N. fischeri* exhibited greater thermal tolerance than *E. repens*. A non-linear interaction between a_w and temperature was observed in *N. fischeri* survival experiments. *N. fischeri* pairwise tests between water activities and temperatures showed significant differences ($P<0.01$) between a_w of 0.70, 0.75 and 0.80 at all temperatures (90°C, 92°C and 94°C) except at 94°C, between 0.75 and 0.80 a_w. *E. repens* demonstrated the greatest thermal tolerance at 0.70 a_w at 78°C (4.873±0.576 min), 80°C (1.645±0.011 min) and 82°C (1.144±0.019 min). In general, the heat tolerance of *E. repens* increased as the water activity decreased however pairwise tests showed no significant difference between *D*-values at 78°C, 80°C or 82°C between 0.75 and 0.80 a_w.

Significance: Our results will be useful for determining limits for thermal processing of low water activity confectionery products to control the growth of osmophilic fungi and to extend shelf life, without the use of preservatives.

P2-09 Heat Resistance of *Salmonella* spp., *L. monocytogenes*, *E. coli* O157:H7 and *E. faecium* on Almonds, Peanuts, Cashews, and Macadamia Nuts

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Introduction: Process validation aimed at addressing *Salmonella* spp. in nuts is an essential part of any food safety plan. Generally, surrogate microorganisms are used during in-plant validations. *Enterococcus faecium* has been determined to be a good surrogate for *Salmonella* spp. on almonds. However, very little data is available to demonstrate that *E. faecium* is an appropriate surrogate for *Salmonella* spp. on other types of nuts. Additionally, it has not been thoroughly investigated if *E. faecium* is an appropriate surrogate for *Listeria monocytogenes* and *Escherichia coli* O157:H7.

Purpose: The purpose of this study was to compare heat resistance of *Salmonella* spp., *L. monocytogenes*, *E. coli* O157:H7, and *E. faecium* on multiple nut types.

Methods: Nuts were evaluated for heat resistance according to the Almond Board of California's "Guidelines for using *Enterococcus faecium* NRRL B-2354 as a Surrogate Microorganism in Almond Process Validation."

Results: Heat resistance of *Salmonella* spp. on almonds, peanuts, cashews, and macadamia nuts showed a 2.88±0.12, 2.57±0.11, 2.75±0.13, and 1.18±0.08 log reduction, respectively. Heat resistance of *E. coli* O157:H7 on almonds, peanuts, cashews, and macadamia nuts showed a 6.76±0.80, 7.40±0.00, 7.77±0.52, and 5.05±0.17 log reduction, respectively. Heat resistance of *L. monocytogenes* on almonds, peanuts, cashews, and macadamia nuts showed a 7.13±0.24, 6.01±0.17, 6.85±0.34, and 6.77±0.07 log reduction, respectively. Heat resistance of *E. faecium* on almonds, peanuts, cashews, and macadamia nuts showed a 1.58±0.28, 3.00±0.25, 1.76±0.09, and 0.68±0.05 log reduction, respectively.

Significance: Heat resistance of pathogens is not uniform across different nut types, and the amount of kill delivered by a lethality process cannot be applied to all nut types. *E. faecium* was shown to be a good surrogate for *Salmonella* spp. on almonds, peanuts, cashews, and macadamia nuts. *L. monocytogenes* and *E. coli* O157:H7 showed significantly lower heat resistance than *Salmonella* spp. and *E. faecium*.

P2-10 The Influence of Water Activity on *Salmonella enterica* Typhimurium Biofilm's Thermal Resistance

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Introduction: Frequent salmonellosis outbreaks and food recalls associated with low water activity (a_w) foods have suggested that they are susceptible to contamination with *Salmonella enterica*. Biofilms are complex bacterial structures characterized by cells embedded in a protective extracellular matrix. Because contamination of dry foods often occurs in processing plant environments, a better understanding of *Salmonella* biofilms is critical for its control.

Purpose: This work was conducted to determine the effect of low water activity on the ability of biofilms of *S. enterica* Typhimurium to tolerate heat exposure.

Methods: Biofilms were grown for 6 days in glass beads in non-selective media at 37°C. After rinsing, beads were dried at 38°C for 4 days and equilibrated at different water activities (0.11, 0.33, 0.53, 1.0) for 7 days. Biofilms were heated at 85 and 95°C and survivors were enumerated after plating in differential media. Scanning electronic microscopy was used to observe cells after thermal treatment.

Results: At low a_w values (0.11, 0.33, 0.53) slight reductions of viability of biofilms that were grown for 2, 4 and 6 days of less than 1 log CFU/g were observed at 95°C after 2 h. At 85°C the same level of viability loss was observed by 5 h. In contrast, the viability of biofilms equilibrated to a_w of 1.0 was reduced by at least 5 log CFU/g after 15 minutes at 85 and 95°C. The structure and morphology of low a_w biofilms was better preserved than that of biofilms exposed to 1.0 a_w as increased amounts of matrix were observed.

Significance: These findings are relevant because biofilms are believed to be the natural form of bacteria occurrence. This work may suggest that in a food processing setting, the exposure of plant environment biofilms to drier conditions may lead to greater persistence.

P2-11 Effect of Thermal Processing on the Survival of *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 in Oats

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Introduction: Recent recalls and outbreaks due to foodborne pathogens in thermally processed low moisture foods highlight the need for food manufacturers to validate their thermal process.

Purpose: The purpose of this study was to evaluate the thermal inactivation kinetics of vegetative pathogens in oats.

Methods: Whole and steel-cut oat samples were inoculated with cocktails of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 to achieve a target level of 10^8 CFU/g. Following inoculation, samples were dried overnight and 25 g were then weighted and vacuum-packed to make a single-layered bag. Sample bags were fully submerged in a circulating oil bath to achieve product temperature of 194°F, 200°F, and 210°F for up to 18 minutes. The oil bath study was conducted to mimic the steam process as a worse-case scenario. Inoculated samples were enumerated for *Salmonella* spp.*, L. monocytogenes,* and *E. coli O157:H7* using scientifically valid methods. The experiment was performed in triplicate. Log transformed data were analyzed using ANOVA to compares the lethality.

Results: Results demonstrated that whole and steel-cut oats processed at 194°F achieved >5-log reductions of *Salmonella* (P<0.5) at 8 and 10 minutes, respectively. For process temperature of 200°F, whole and steel-cut oats achieved >5-log *Salmonella* reductions (P<0.5) at 4 and 5 minutes, respectively. *L. monocytogenes* and *E. coli* O157:H7 in whole and steel-cut oats processed at 194°F achieved >5-log reductions within 2 minutes (except for *E. coli* O157:H7 in steel-cut oats at 4 minutes). Whole and steel-cut oats achieved >5-log reductions of *Salmonella* spp., *L. monocytogenes,* and *E. coli* O157:H7 at 210°F within 30 seconds.

Significance: The study indicates that the thermal process employed by the oat manufacturing facility achieves an acceptable lethality of vegetative pathogens. The data generated in this study provides scientific basis to support the steam process as a validated microbial lethality step.

P2-12 Effect of Oil Roasting on *Salmonella enterica* Serovar Enteritidis PT30 on Coated Almonds

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Introduction: Almond Board of California (ABC) previously evaluated the efficacy of hot oil to reduce *Salmonella* Enteritidis PT30 on oil-roasted almonds and determined that a minimum process of 2.0 minutes at 260°F or above is required to provide a 5-log reduction of *Salmonella* on whole kernel almonds. ABC would like to determine if the oil roasting process recommended for whole kernel almonds is suitable for various sugar coated almonds which are also produced under similar process conditions.

Purpose: To evaluate the impact of different almond coatings (Honey, Xanthan, Starch) on the effectiveness of oil roasting at 260°F for various holding times to kill *Salmonella* on almonds.

Methods: Whole kernel almonds were artificially-inoculated with *Salmonella*, coated with a Honey, Xanthan, or Starch-based coating, and then oil-roasted at 260°F for 0.0, 1.0, 1.5, 2.0 minutes. Inoculated uncoated almonds were also included in the study for comparison. Log reductions following heat treatment for each treatment time between each almond product were compared using ANOVA.

Results: Log reductions of *Salmonella* following heat treatment at 260°F for 1 or 1.5 minutes were inconsistent, and ranged between 3.08±0.93 and 4.89±1.73 logs (n=9), or 4.87±0.38 and 6.28±0.90 logs (n=9), respectively, with standard deviations ranging from 0.35-1.73 log CFU/g for each of the different almond products. A greater than 5-log reduction of *Salmonella* was achieved after oil roasting at 260°F for 2.0 min, with the least amount of reduction observed on the uncoated almonds. No significant differences (P>0.05) were observed between the log reduction of *Salmonella* achieved for each type of sugar-coated almond after exposure to hot oil for 1.5 or 2.0 minutes.

Significance: The commercial oil roasting process at a higher temperature of 260°F for longer than 2.0 minutes should be sufficient to achieve a greater than 5-log reduction of *Salmonella* on Uncoated, Honey Coated, Xanthan Coated, and Starch Coated almonds.

P2-13 Effect of Product Structure and Water Activity on X-ray Inactivation of *Salmonella* in Low-water Activity Foods

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Introduction: *Salmonella* contamination of low-moisture foods is a socioeconomically important issue evidenced by the multiple outbreaks and recalls in recent years. Thus, it is critical to develop effective mitigation strategies to significantly reduce foodborne pathogens while maintaining the integrity of the food product.

Purpose: The purpose of this study was to assess the efficacy of X-ray irradiation in inactivating *Salmonella* in almonds (kernels, meal, butter), dates (whole fruit, paste), and wheat (kernels, flour) at various water activities (a_w).

Methods: Almond kernels were inoculated with *Salmonella* Enteritidis PT30, conditioned to 0.25, 0.45, or 0.65 a_w, and ground into coarse meal. Dates were spot inoculated on the surface of the whole date and conditioned to the target a_w. Bagged samples (~1-4 g) were irradiated utilizing a 70 kV X-ray irradiator at doses targeting 1-5 log reductions. Samples were then stomached, diluted, and plated on modified tryptic soy agar, and the survival counts were used to determine radiation D_{10}-values by linear regression.

Results: D_{10}-values for whole dates (2.17 and 2.03 kGy at 0.45 and 0.65 a_w, respectively) were unexpectedly insensitive to a_w (P = 0.64). However, the D_{10}-values for almond products were significantly (P < 0.05) lower than for the dates (e.g., 0.51 kGy for 0.45 a_w almonds and 0.74 kGy for 0.65 a_w almond meal).

Significance: As a nonthermal intervention technology, X-ray irradiation is a viable tool that can effectively reduce bacterial population in low-moisture food products without significant impact on overall food quality, as shown in a prior study. However, the effects of product structure and a_w are complex and probably need to be considered on a case-by-case basis.

P2-14 Inactivation of Pathogens on Peppercorns and Sunflower Kernels Using a Pilot Scale Vacuum Steam Pasteurization System

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Introduction: Spices, seeds and grains are some of the low moisture foods that have been implicated in foodborne outbreaks due to *Salmonella* and *E. coli* O157:H7. It is important to investigate inactivation methods to eliminate risks due to pathogens in such foods with minimum impact on product quality and functionality. Vacuum steam pasteurization (VSP) utilizes steam under vacuum which can be operated at temperatures below 100°C.

Purpose: We wanted to quantify inactivation of pathogens on peppercorns and sunflower kernels and also determine if *E. faecium* can be used as a potential surrogate for *Salmonella* and *E. coli* O157:H7 when using VSP.

Methods: Sunflower kernels were separately inoculated with *Salmonella* PT30, *E. coli* O157:H7 and *Enterococcus faecium*, and peppercorns were inoculated with only *Salmonella* PT30 directly with bacterial lawns grown on brain heart infusion agar plates and suspended in 5 ml of water. Following equilibration of a_w to initial levels, 25 g of inoculated samples were pasteurized for varying times (0.5-5.0 min) at 75, 85, 95, and 105°C in triplicate.

Results: Treatment of peppercorns using VSP yielded greater than 5 log CFU/g reductions of *Salmonella* PT30 after just 30 seconds at 75°C. For sunflower kernels, average reductions of 5.09 ± 0.92 log CFU/g was observed for *Salmonella* PT30 after 4 minutes at 75°C, 5.40 ± 0.83 log CFU/g for *E. coli* O157:H7 after 1 minute at 75°C, and 5.69 ± 0.94 log CFU/g for *E. faecium* after 2 minutes at 85°C. However, similar log reductions (>6.0) were observed after treatment at 95 and 105°C.

Significance: VSP can be effectively used to reduce pathogens in peppercorns and sunflower kernels at temperature as low as 75 and 85°C and *E. faecium* may be used as a potential surrogate for *Salmonella* PT30 and *E. coli* O157:H7 with careful considerations of treatment time and temperatures.

P2-15 Resistance of Spice-related *Salmonella* Serotypes and *Pediococcus faecium* NRRL B-2354 to Dehydration, Gamma-irradiation and Dry Storage

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Introduction: Using spice-related *Salmonella* strains (SRSS) is preferred when designing validation studies for treatments to control *Salmonella* in spices. Non-spice-related strains (non-SRSS) may not accurately represent potential adaptation to dry environments likely occurring in SRSS. Studies should also include selecting surrogates for in-plant validations.

Purpose: To test resistance of SRSS and non-SRSS and of *Pediococcus faecium* as potential surrogate, to desiccation, irradiation and dry storage in talcum powder as dry-inoculation vehicle for spices.

Methods: Talcum powder was inoculated with cocktails containing approx. 10 log CFU/ml of: 1) *Salmonella enterica* ser. Rissen SAL1449 and Montevideo SAL4599 (RM), 2) *S. enterica* ser. Choleraeasuis ATCC-13312, Enteritidis ATCC-4931, Newport ATCC-6962, and Typhimurium ATCC-700720 (MP15), or 3) *Pediococcus faecium* NRRL B-2354 (PF). These inoculated talc batches were dried at 35°C until reaching 0.45 a_w. The desiccation effect was determined by counting surviving *Salmonella* and PF. Sample dilutions were surface-spread onto TSA, incubating at 35°C for 3 h and then overlaid with XLT4 or KFS to recover stressed bacteria, continuing incubation for 24 h. Inoculated talc sets were gamma-irradiated and D_{10}-values were calculated. Survivability was determined by storing the talc at 25, 4, and -18°C, determining bacterial populations at intervals during 15 weeks.

Results: Counts of RM and MP15 were reduced by 2.1 and 3.4 log cycles, respectively, during talcum drying (P<0.05), whereas PF was reduced 0.1 log. RM, MP15 and PF showed no significant differences over the 15-week dry storage at -18, 4 and 25°C (P<0.05). D_{10}-values for RM and MP15 were 0.673 and 0.623 kGy, respectively, (P>0.05), whereas for PF was 0.743 kGy (P>0.05).

Significance: *Salmonella* strains isolated from spice-related outbreaks survived dehydration better than non-spice-related strains during inoculum preparation. PF was more resistant than *Salmonella* to dehydration, irradiation and storage in dry conditions, supporting its potential use as surrogate.

P2-16 Radio Frequency Pasteurization of Peanut Butter: Quality Evaluation

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Introduction: The low thermal conductivity of peanut butter creates a significant challenge in developing a pasteurization process that sufficiently inactivates *Salmonella*. In addition, *Salmonella* is heat-resistant in low water activity foods such as peanut butter. Alternative methods such as radiofrequency (RF) heating have the potential to overcome the limitations of conventional thermal processing. However, any form of thermal processing could degrade the quality of food.

Purpose: This study aims to investigate the effects of an RF heating pasteurization process on the quality of peanut butter.

Methods: Thermal treatments for the peanut butter (83°C for 110 min and 90°C for 49 min) were chosen based on *Salmonella* thermal inactivation studies in the literature. The quality parameters measured include fatty acid composition, peroxide value, volatile compounds, oil separation, color, spreadability, and water activity. Quality evaluations were done immediately after RF heating, after a month of storage at 23°C, and after a month of accelerated storage (40°C) in triplicates.

Results: The spreadability (hardness and adhesiveness) of RF heated peanut butter was not significantly (P-value < 0.05) different from the control for all storage conditions. The largest color difference, ΔE (versus control) was 1.6, which would be hardly discernible by consumers. Oil separation was unaffected (P-value < 0.05) by RF heating, although accelerated storage induced oil separation in all samples. Four phytosterols (campesterol, stigmas-

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terol, β-sitosterol and Δ^5-avenasterol) were identified in the samples and two of them (campesterol and stigmasterol) were significantly (*P*-value < 0.05) higher in peanut butter treated at 83°C for 110 min, regardless of storage time. The remaining quality analyses are being performed, but results thus far suggest that RF heating can pasteurize peanut butter without significant quality degradation.

Significance: These results can serve as guidance for determining the appropriate thermal intensity of pasteurization processes for inactivating *Salmonella* in peanut butter.

P2-17 Evaluation of Water Content as a Convenient Metric in Thermal Inactivation Modeling for Low-moisture Foods

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Introduction: FSMA Preventive Controls Rules require validation of pathogen reduction steps in the food industry, which increases the importance of microbial modeling for process validations. Water activity (a_w) has been the most commonly used metric when evaluating and modeling the effect of water on *Salmonella* inactivation in low-moisture foods. However, because of the nature of these products and processes, as well as the definition of a_w (a function of temperature), it may not be the most suitable metric for real-world applications.

Purpose: The objective was to quantitatively compare the correlation of moisture content (%mc) and a_w with *D*-values, to evaluate the utility of %mc as a metric in pathogen inactivation models and industrially-relevant process validation protocols.

Methods: $D_{80°C}$ values for *Salmonella* Enteritidis PT30 were calculated by linear regression of isothermal inactivation data from multiple related studies in our laboratory (wheat flour, almonds, dates; 0.25, 0.45, 0.65 a_w; 70-90°C, triplicate). Water activity and %mc were measured and/or calculated from moisture isotherms (sorption and desorption, accordingly). Linearity of inactivation curves was confirmed, and correlation coefficients were estimated between logD and a_w and %mc.

Results: The $D_{80°C}$ values for the different products exhibited a log-linear trend with a_w, as well as with %mc. The correlation coefficients varied less than 5% when comparing a_w and %mc vs. logD (e.g., -0.96 and -0.95, respectively, for wheat flour). The results suggest that %mc may be a suitable, or even preferable, metric for the effect of water on inactivation process.

Significance: When comparing the utility of a_w vs. %mc for inactivation modeling or process validation, %mc has the advantage of being measurable (potentially real-time in dynamic processes), and this study shows a consistent correlation with logD. This is critically important to both monitoring and modeling inactivation processes in low-moisture foods.

P2-18 Scalability of a Discrete Element Model for *Salmonella* Cross-contamination in Granular Low-water Activity Foods

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Introduction: Modeling cross-contamination of bacteria in granular low-moisture foods is a particular challenge due to the discrete nature of these materials. Scaling-up models based on laboratory data to industrial-scale system is limited because of the lack of first-principle models. An ideal cross-contamination model should enhance its scalability, so that it can be utilized for an industrial-scale system without further validation burden.

Purpose: The purpose of this study was to assess the scalability of a discrete element method (DEM) model of bacterial cross-contamination to industrial-scale systems.

Methods: Almond kernels were inoculated with *Salmonella* Enteritidis PT30 and mixed with clean almonds in a rotating drum at a bench top scale of ~200 g (5 g of inoculated almonds). A DEM bacterial transfer model was developed from these results and validated against a pilot-scale model of ~1 kg. After validation, the model was used to simulate an industrial-scale scenario of ~200 kg of almonds mixed with 5 kg of contaminated almonds.

Results: The lab-scale experiments (with contaminated almonds at ~8.3 log CFU/g) yielded 4.3±0.2 log (CFU/g) maximum transferrable bacterial load after 600 s at 8 rpm. The calibration model of the experiment was fit to the data (RMSE=0.005 log CFU/g) and validated with pilot-scale data sets (RMSE=0.057 log CFU/g). The results for the 200 kg rotary batch mixer simulated a similar trendline as actual experiments, showing a maximum transferrable bacterial load of 4.1±0.1 log (CFU/g) after 600 s at 8 rpm, and demonstrated reasonable scalability of the DEM model.

Significance: DEM modeling appears to be an efficient tool to model the interactions of particulate low-moisture food products. The scalability of the DEM model will contribute to risk modeling associated with bacterial cross-contamination scenarios.

P2-19 Modeling the Effect of Product Temperature, Moisture, and Process Humidity on Thermal Inactivation of *Salmonella* in Pistachios

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Introduction: The 2015 Preventive Controls for Human Foods Rule requires firms to validate pathogen-reduction steps. Some thermal processes, such as pistachio roasting, are not yet well characterized with respect to the impact of product and process variables on *Salmonella* lethality.

Purpose: The objective was to quantify the effect of product temperature, product moisture, and process humidity on *Salmonella* lethality on in-shell pistachios.

Methods: In-shell pistachios were inoculated with *Salmonella* Enteritidis PT30 (~8.5 log CFU/g), equilibrated in controlled-humidity chambers (0.45 or 0.65 a_w), and, in some cases, exposed to a pure-water or 27% NaCl brining treatment for 30 s (0.95 and 0.75 a_w, respectively) prior to thermal treatment. Samples (15 g) were heated in a computer-controlled, laboratory-scale, moist-air convection oven, following a full-factorial experimental design (in duplicate) with process temperatures of 104.4 and 118.3°C, process humidities of ~3, 15, and 30% v/v (corresponding to dew points of ~23.8, 54.4, and 69.4°C, respectively), and air speed of 1.3 m/s. *Salmonella* survivors, moisture content, and a_w were quantified at six time points during each treatment, targeting cumulative lethality of ~3 to 5 log. Inactivation rates were modeled as a function of time, product temperature, product moisture, and process dewpoint.

Results: Increasing product temperature or process dewpoint increased *Salmonella* inactivation rates ($P<0.05$). For unbrined and brined treatments, analyzed separately, initial product a_w did not affect inactivation rates ($P>0.05$). However, when comparing unbrined against brined treatments, inactivation rates were greater ($P<0.05$) for the brined pistachios.

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Significance: Product and process moisture both appear to impact pathogen reduction in low-moisture products, and this project quantifies those impacts for *Salmonella* inactivation in pistachios. Considering these factors is critically important when designing and validating thermal processes as pathogen-reduction steps.

P2-20 Factors Affecting Bacterial Cross-contamination Using *Salmonella* and a Surrogate Organism during Almond Processing

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Introduction: Outbreaks and recalls associated with *Salmonella* in low water activity foods suggest a need for improved understanding of factors contributing to pathogen transfer and cross-contamination with these types of products, including water activity (a_w), bulk handling parameters, and mode of transfer.

Purpose: The objective was to compare inoculation method and the use of *Enterococcus faecium* as a surrogate for *Salmonella* cross-contamination using almonds at different water activities and rotational speeds in a rotating drum.

Methods: Almond kernels (200 g) were wet- and dry-inoculated with *Salmonella* Enteritidis PT30 or *Enterococcus faecium*. The inoculated kernels (5 g, ~8 log CFU/g) and un-inoculated kernels (200 g) were conditioned to 0.2, 0.4, or 0.6 a_w and tumbled in a stainless steel drum (140 mm diameter, 64 mm depth) for the total number of rotations (TNR) of 5, 20, 40, and 80 rev (three rotational speed of 8, 16, and 24 rpm × durations (10-300 s)), in triplicate. At each condition, samples (four kernels) were retrieved, stomached, plated on modified trypic soy agar, and incubated to enumerate transferred bacteria.

Results: Water activity significantly affected maximum transferred bacterial load (MTBL) for wet inoculation method ($P < 0.001$), but not for dry inoculation methodology. For lower water activity levels (0.2 and 0.4 a_w), choice of inoculation methodology significantly affected MTBL ($P < 0.001$), but not at 0.6 a_w ($P = 0.10$). *E. faecium* may not be a surrogate for *Salmonella* at 0.4 a_w ($P = 0.04$), but needs further verification.

Significance: Knowing how environmental/physical conditions influence bacterial transfer will enhance the accuracy of cross-contamination modeling, in terms of secondary models, and contribute to elucidating mechanism of cross-contamination in low-moisture foods.

P2-21 The Effect of Corn Oil as an Additive to Sequester Phenolic Compounds in Spices, and Increase *Salmonella* Recovery: A Comparison between TSB and mBPW

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Introduction: Oregano essential oils and phenolic compounds such as carvacrol and thymol inhibit the detection of pathogenic microorganisms in spices. In this study, corn oil is added to the pre-enrichment broth as an additive to neutralize the effect of these phenolic compounds.

Purpose: The increased recovery of *Salmonella* was evaluated by comparing two pre-enrichment broths: modified Buffer Peptone water (mBPW) and Trypticase Soy Broth (TSB).

Methods: Oregano samples (25 g) were artificially contaminated with 10^3 lyopholized *Salmonella* serovar Montevideo and aged for 2 weeks. Oregano samples were pre-enriched in mBPW and TSB with and without 2% (vol/vol) corn oil, respectively, and incubated overnight at 37°C. Samples were transferred to selective enrichment broths Rappaport-Vassiliadis (RV) and tetrathionate (TT) and plated on Xylose-Lysine-Tergitol 4 (XLT-4) agar at 24 h. Typical *Salmonella* colonies were examined as described in the FDA Bacteriological Analytical Manual (BAM), and suspect colonies were confirmed using the Vitek® 2 Compact.

Results: The samples with corn oil had ≥10 fold more recovery than the samples without the corn oil. The average recovery of *Salmonella* was 323 CFU with corn oil compared to 24 CFU without corn oil. There were no significant differences between mBPW and TSB, thus the results suggest that the increase in recovery was due to the corn oil.

Significance: Detection of pathogens from oregano samples is repressed by the release of the phenolic compounds, so finding an approach to increase recovery and neutralize the effect of the antimicrobial phenolic compound is a key for a detection method.

P2-22 An Independent Evaluation of Alternative Rapid Methods for the Detection of *Salmonella* in Select Emulsifier Ingredients

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Introduction: *Salmonella* outbreaks from low moisture products ($a_w < 0.85$) are relatively rare but often impact large numbers of people. In recent years, a number of these outbreaks has illustrated the point that, while this organism cannot grow in these products, it does have the ability to persist for long periods of time and still cause illness due to its low infectious dose. Many rapid assays have been validated for the detection of *Salmonella*; however, these validations do not typically include a wide range of low moisture products. Recent outbreaks highlight the importance to public health that rapid methods are validated for these commodities.

Purpose: To conduct an initial feasibility evaluation of the performance of AOAC OMA 2013.03 and 2003.09 for ten emulsifiers. A larger method validation study was conducted on one emulsifier upon completion of the feasibility study.

Methods: Feasibility Study: For each matrix, 5 replicates at a high contamination level (5-10 CFU/test portion) were evaluated by the alternative RT-PCR methods. Method Validation: Thirty sample replicates (20 low level contamination, 5 high level contamination, 5 uninoculated control) were evaluated in a paired study by the alternative methods and the FDA/BAM Chapter 5.

Results: Feasibility Study: No issues were observed for 9 of 10 emulsifiers. For one matrix, Panodan 150 LP, no recovery of the analyte from the reference method was observed. Further analysis resulted in a modification to the enrichment media to improve recovery. This matrix was selected for the full validation. Using POD, no statistical significant difference was observed between the alternative and reference method.

Significance: The data generated in this evaluation indicates that the rapid alternative methods with a modified enrichment media containing a surfactant are a suitable alternative method for the detection and recovery of *Salmonella* in these select commodities.

Poster

P2-23 An Independent Evaluation of RapidChek *E. coli* O157 (including H7) Test Kit for the Detection of *Escherichia coli* O157:H7 in Select Ready-to-Eat Meats

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Introduction: In the United States, *E. coli* O157 is the most prevalent of the pathogenic *E. coli*, contributing to an estimated 265,000 infections per year. *E. coli* O157:H7 can cause hemorrhagic colitis, which manifests as severe cramping causing abdominal pain, diarrhea, and can lead to kidney failure or death. It is critical for the food industry to have methods that can rapidly detect the organism, yet are sensitive enough to detect the pathogen in low quantities. The RapidChek *E. coli* O157 (including H7) offers the benefits of a single, proprietary enrichment, coupled with innovative immuno-strip technology to allow processers and manufacturers the ability to detect the dangerous pathogen from ready-to-eat meats in as few as 8 h.

Purpose: The purpose of this independent evaluation was to compare the new method to the USDA/FSIS-MLG 5.09 reference method for select ready-to-eat (RTE) meats: deli ham, bacon, all beef hot dogs, salami, and pimento loaf.

Methods: Using unpaired samples, the new method was evaluated using 25- and 75-g test portions. For each food matrix, 20 test portions were inoculated at a low inoculation level of 0.2 to 2 CFU/test portion and evaluated along with 5 uninoculated control test portions. After sample enrichment in RapidChek *E. coli* O157 enrichment media, test portions were evaluated after 8 and 24 h of incubation (18 and 24 h for salami). Samples were confirmed following procedures outlined in the USDA/FSIS-MLG 5.09.

Results: Results for each assay were compared to the MLG method by POD statistical analysis. No statistically significant differences were observed between the new method and the reference method.

Significance: This new method demonstrated reliability as an easy to use, rapid method for the detection of *E. coli* O157:H7 in select ready-to-eat meats.

P2-24 Rapid Detection of Microbial Contamination in UHT Beverages Using Microbial Luminescent Technology

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Introduction: Beverages processed with ultra-high temperature treatments (UHT) or extended shelf life (ESL) have increased demand in the food market. Microbial analysis for testing commercial sterility is traditionally done with agar plating after product enrichment, which can take several days to confirm a negative result delaying product release.

Purpose: To evaluate a novel universal rapid detection kit based on microbial ATP bioluminescence for screening commercial sterility compared to traditional methods including growth on agar and pH measurement.

Methods: A variety of twenty-one UHT or ESL beverages (*n*=2/beverage/microorganism tested) were inoculated with 10 to 100 CFU/container utilizing a panel of microorganisms including a combination of bacteria (gram positive, gram negative and lactic acid bacteria), yeast and molds as applicable. A negative control (uninoculated blank) and positive control (inoculated with ~1,000 CFU) were also included in each matrix (N=1,104 total samples). Inoculated beverage containers were pre-enriched at 30°C for up 48 and 72 hours for dairy beverages and juices or juice dairy combinations, respectively. After enrichment beverages were screened for commercial sterility utilizing standard methods agar (SMA) as a reference method, pH and a novel universal Microbial Luminescence System based on the detection of microbial ATP.

Results: Utilization of microbial ATP bioluminescence resulted in a rapid method to screen commercial sterility in UHT beverages. Sensitivity and specificity were 98 and 99%, respectively, compared to agar. The study demonstrated that the novel universal kit can detect the presence of microbial contamination > 48 hours sooner than the traditional agar method. The study also demonstrated that pH is not a reliable parameter to screen for commercial sterility as not all microorganism will modify the pH during pre-enrichment.

Significance: Microbial ATP bioluminescence can provide a rapid result to screen commercial sterility by significantly reducing time to product release and thus reducing inventory hold times.

P2-25 Aerobic Plate Count Media Repeatability Comparison

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Introduction: Microbiological media is an important aspect of proficiency testing and daily testing of routine samples. Variables such as lot, formulation and preparation of microbiological media can affect plate count repeatability and consistency.

Purpose: The purpose of the study was to compare plate-to-plate count variation between four different aerobic count media manufacturers.

Methods: One lot of Standard Methods Agar (SMA) from three global dehydrated media manufacturers and a rehydratable film media, 3M Petrifilm Aerobic Count (AC) Plate, were compared. A multi-organism certified reference material containing four microorganisms was used to prepare the inoculum in three diluents. Plates were incubated at 30°C, 32°C or 35°C for inoculum prepared in Peptone Saline Buffer (PS), Butterfield's Phosphate-Buffered Dilution Water (BPB) or Buffered Peptone Water (BPW), respectively. Following incubation, plates were counted and results were recorded. The experiment was repeated three times with 10 replicates for each agar media brand.

Results: Plate count variance, based on the standard deviation between replicates (*n*=30), of each agar media brand were compared to determine any significant differences (*P*-value < 0.05) between them. The rehydratable film media had the lowest standard deviation for plates incubated at 30°C and 32°C. Agar media A had the lowest standard deviation for plates incubated at 35°C. However, the standard deviation value was not significantly different than the standard deviation for the rehydratable film media. All agar media brands incubated at 32°C had significantly higher standard deviations than that of the rehydratable film media. Agar media B, when incubated at 30°C, had a significantly different standard deviation than the rehydratable film media.

Significance: Repeatability from microbiological media is an important component when conducting proficiency test and testing routine samples. The rehydratable film media had statistically equivalent or better plate-to-plate repeatability than three global manufacturers of SMA dehydrated media.

P2-26 Performance Characteristics of a Rapid Microbial Detection Technology

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Introduction: A laboratory study was conducted to evaluate the key performance parameters for the GreenLight® rapid microbial detection system. This data could then be used to predict performance in food matrices and allow users to determine the best fit to their application.

Purpose: The study sought to determine the accuracy, limit of detection, limit of quantification, and linearity of up to 4 sample bacterial species on the rapid instrument. All tests were performed in an independent ISO17025-certified test laboratory and compared to FDA BAM equivalent methods for Aerobic Plate Count (APC).

Methods: Parallel tests were performed on the rapid instrument and standard plate counting methods. All samples were single ATCC bacterial strain inocula into Tryptic Soy Broth (TSB). The strains used were *E. coli* 25922, *L. innocua* 33090, *P. aeruginosa* 10145, and *S. aureus* 25923. Serial dilutions for a single strain or a cocktail of strains from 10^7 CFU to 10^1 CFU in 5 replicates where made and the results tabulated for each performance parameter.

Results: Accuracy of a 4-strain cocktail compared to standard plating methods was found to be above 90%. There was a coefficient of linearity of (R^2) = 0.9533. The limit of detection for *P. aeruginosa* was 0.87 CFU/ml and the same organism gave limit of quantification of 8.7 CFU/ml, both over the range 2 to 7 log CFU/ml.

Significance: The results reveal that this rapid system can be used at very low levels of detection when classical enumeration methods are ineffective. Therefore, the system can enhance quality control by correctly flagging more positives while reducing time-to-result.

P2-27 Intralaboratory Evaluation and Selection of Total Aerobic and Coliform Count Methods

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Introduction: Many food products, including Grade "A" milk, are required to be tested using validated methods. Accredited milk laboratories must use approved methods, however, there is limited guidance on method selection when multiple, validated methods are available for detection or enumeration of a single analyte.

Purpose: An intralaboratory study evaluating four aerobic count methods and five coliform count methods was conducted to outline an example approach for method selection. Statistical analyses were used to analyze data from each method and a number of testing characteristics (cost, time, incubation temperature, etc.) were used to compare testing methods.

Methods: A cocktail of typical milk isolates was used to inoculate various milk products. Testing data for aerobic counts in ten blind replicate samples and coliform counts in five blind replicate samples were collected by trained, experienced laboratory analysts. Statistical analyses included computation of robust mean, standard deviation, bias, and Mandel's h and k statistics.

Results: Total aerobic counts across four methods had mean biases ranging from -0.17-0.14 log CFU/ml, while mean bias for coliform count data from five methods ranged from -0.32-0.21 log CFU/ml. Reproducibility standard deviations ranged from 0.04-0.14 log CFU/ml for aerobic counts and 0.05-0.26 log CFU/ml for coliform counts. Cost, preparation time, incubation time and temperature, and limit of quantification were summarized for all methods used. There was no one method that was ideal across all testing characteristics for either analyte.

Significance: This study outlines an example of a practical approach to conduct a method selection study within a single laboratory. The steps taken in this study demonstrate that methods, even when validated, have unique considerations and drawbacks. The process utilized in this study summarizes one way for laboratories to make decisions regarding method selection.

P2-28 Direct and Conventional Multiplex PCR Assays to Detect the Zearalenone Producing Fusarium Species in White and Brown Rice

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Introduction: Zearalenone (ZEA) is an estrogenic mycotoxin produced by some species of *Fusarium* and commonly found in rice throughout the world.

Purpose: The aim of this study is to detect ZEA producing *Fusarium* species in white and brown rice by conventional and direct multiplex polymerase chain reaction (mPCR).

Methods: The presence of 4 genes (*PKS4, PKS13, ZEB1* and *ZEB2*) involved in ZEA biosynthesis was tested for 15 referenced *Fusarium* strains to optimize conventional and direct mPCR assays (in terms of specificity and sensitivity). Chemical analysis of ZEA was carried out for *Fusarium* cultures and rice samples by high performance-liquid chromatography. To check the practical usefulness of two mPCR assays, 51 *Fusarium* strains were evaluated by conventional mPCR, and artificially inoculated (10^1-10^6) and naturally contaminated rice (*n*=41) were evaluated by direct mPCR.

Results: The conventional mPCR was highly specific in detecting ZEA producing species containing these genes and was sensitive, detecting up to 1.25 pg/ml of genomic DNA. Direct mPCR without the necessity of time-consuming DNA isolation was shown to detect the presence of ZEA producing species at the lowest level of 10^1 macroconidia/g of white rice and 10^2 macroconidia/g of brown rice.

Significance: These results suggest that both mPCR methods are suitable for the specific detection of ZEA producing *Fusarium* species in white and brown rice, but direct mPCR gives faster results.

P2-29 Evaluation of 3M Petrifilm Rapid Aerobic Count Plate for Enumeration of Aerobic Microorganisms in Thailand Seafood Products

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Introduction: The aerobic plate count (APC) is used to determine the level of microbial burden in foods. When used on seafood products, generally APC does not relate to specific food safety hazards, but it is useful as a quality, shelf life, and post-contamination indicator. Seafood processing plants utilize APC as part of their quality management program to test a large variety of samples including raw materials and finished goods.

Purpose: This study aimed to compare the performance of the 3M Petrifilm Rapid Aerobic Count (RAC) Plate at 32°C and 35°C with the performance of the U.S. Food and Drug Administration Bacteriological Analytical Manual (FDA-BAM) reference method for the enumeration of aerobic organisms in seafood products from Thailand.

Methods: The method comparison was conducted using naturally contaminated seafood matrices (*n*=55) comprised of (32) fish (17) shrimp and (6) cephalopod samples. Each seafood sample was homogenized and 1 ml of the homogenate was serially diluted in Butterfield's phosphate buffer. Samples (1 ml) were placed onto the RAC Plate and the reference method (FDA-BAM) plates. Colonies were counted after a 24 ± 2-hour incubation at 32 ± 1°C and 35 ± 1°C for RAC plates and a 48 ± 3 hours incubation at 35 ± 1°C for the FDA BAM reference method and the results were compared.

Poster

Results: Coefficients of determination (R^2) of 0.9878 and 0.9822 for RAC plates incubated at 32°C and 35°C, respectively, and the reference method indicate a high degree of correlation between both methods.

Significance: 3M RAC plate provided ease of use and addresses the need for faster enumeration of total aerobic microorganisms in seafood products. RAC plates provided results in 24 hours or half the time it takes the for the reference method to produce counts at 48 hours.

P2-30 Performance Assessment of a Rapid Microbial Screening Tool in a Slovakian Meat Processor

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Introduction: Many national cultures promote the use of fresh local ingredients and short time-to-market. Meat processors must meet the challenge of controlling quality from several suppliers while continually avoiding food safety issues. This study assessed the performance of a novel rapid method for Total Plate Count as a supplier screening tool.

Purpose: A novel oxygen depletion technology, GreenLight, has seen increasing use as a screening tool for fresh food. This technology uses the oxygen respiration of viable aerobic organisms to rapidly estimate the Total Plate Count (Total Viable Count) of food samples. An automated system was used in a plant trial in Slovakia over a period of 4 months, to measure and improve the incoming quality of raw pork meat, as measured by TPC.

Methods: The study assessed incoming lots of pork meat from up to 5 suppliers, using the rapid system calibrated for results in CFU/g. Each sample was weighed and diluted following the preparation guidelines for ISO4833:2013. However, no serial dilutions or fixed incubation periods were required. **Results** for the overall period were tabulated to determine average counts, pass/fail and supplier trends.

Results: One hundred twenty-two (122) pork samples were tested over 4 months. The producer selected an automatic "fail" level of 1 million CFU/g (6 log CFU/g). In the evaluation period, 13 samples failed these criteria (10.7%). The mean result from the rapid system was 5.2 log CFU/g. Failed samples were identified by unique bar codes. The technology returned lower test times the higher the microbial load; therefore it was possible to predict a maximum assay period by which 99.9% of samples would show a countable result. This was found to be 8 hours.

Significance: The study predicts that this meat producer can identify TPC results within 8 hours, allowing rejection of incoming materials, identifying offending suppliers and rewarding compliant suppliers.

P2-31 Detection of Multiple Foodborne Pathogen Genera in a 96-Well Assay at Ten CFU/g Food within Five Hours

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Introduction: While polymerase chain reaction analyzers are replacing culture growth to reduce foodborne pathogen analysis times, they are only effective if there are 10,000 to 100,000 pathogenic cells per gram of sample. For this reason numerous samples are "pooled" together and added to enrichment broths to achieve such concentrations. Not only does analysis still take 20 to 30 hours, but if a sample tests positive, all of the individual samples (10 to 25) must now be analyzed.

Purpose: There is a critical need for a *multiplexable* analyzer that can rapidly detect foodborne pathogens at 1 CFU per gram of food in multiple samples simultaneously, in a few hours (not days).

Methods: Twenty-five gram food samples were inoculated with 1 of 3 pathogen genera at 10, 100, or 1,000 CFU/g, then incubated for 4 hours in an enrichment broth. Filtered samples were then added to antibody functionalized 96-well microplates, which were washed to remove unbound materials. Silver colloids were then added to the wells to produce surface-enhanced Raman spectra (SERS) using a Raman spectrometer.

Results: Samples of 10 CFU/g *Campylobacter jejuni* in chicken, *Listeria monocytogenes* in cheese, and *Salmonella* Typhimurium in chocolate were detected within 5 hours using this assay. Measurements of each pathogen genera in wells functionalized with the antibodies specific to the other two genera required concentrations at least 1,000 times greater to produce a SERS signal.

Significance: The entire time required to detect foodborne pathogens in food or equipment samples at 10 CFU, can be performed in 5 hours, which includes 4 hours of broth enrichment. As many as 96 samples can be measured simultaneously, allowing 1) the detection of multiple pathogen genera and species, 2) analysis of multiple sample sources, 3) repeat measurements, and 4) reference measurements of control samples.

P2-32 Performance Evaluation of MilliporeSigma *ReadyPlate 55 Chromocult Coliform Agar (CCA) ISO 9308* and *EZ-Pak Membrane Filters* for Membrane Filtration Applications, in Compliance with the New ISO 11133:2014 Standard and ISO 9308-1:2014

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Introduction: To detect *Escherichia coli* and coliform bacteria in water, ISO 9308-1:2014 specifies membrane filtration and Chromocult Coliform Agar (CCA). ISO 11133:2014 is mandatory for the culture media, requiring validation of EACH membrane filter batch with EACH culture media batch, considerably increasing workload and costs for testing laboratories.

Purpose: A membrane filtration comparative study to demonstrate performance of different CCA brands in combination with matched membrane filters, according to ISO 11133:2014 and a shelf-life study using stressed CCA agar (72°C, 40 h), to demonstrate stability at 25°C and 60% humidity for 6 months minimum.

Methods: ISO 9308:2014 control strains selected: 3 for shelf-life and 4 for comparative. One hundred ml 0.9 % NaCl, containing the test organism, was filtrated according to ISO 11133:2014 using filters from own brand, Competitors A and B (comparative study only) and non-selective agar as reference. For shelf life, CCA was first stressed for 40 h at 72°C and stored at 25°C, 60 % humidity for at least 6 months. Productivity calculations were conducted according to ISO 11133:2014 with CCA acceptance criteria of ≥70%, according to ISO 9308-1.

Results: All CCA brands complied with the productivity specification of ≥70 % for all control strains. Productivity rates for Competitors A and B were lower than own brand CCA rates. Membrane application and removal on/from the media was more difficult with Competitors A and B. Shelf life demonstrated up to 6 months.

Significance: ReadyPlate 55 CCA 9308 and EZ-Pak Membranes ISO 9308-1:2014 and ISO 11133:2014 compliant kits for water testing filtration, optimize incoming QC costs of filter/media batch combination and reduce refrigeration storage. This solution is aimed at streamlining the ISO 17025 accredited laboratories incoming workflow, and increasing the technicians' peace of mind.

Poster

P2-33 Performance Evaluation of 3M Petrifilm RAC for Rapid Aerobic Counting on Brazilian Beef Matrices

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Introduction: Microbial contamination of fresh meat can occur during various steps in the manufacturing process. Aerobic plate counts provide important overall quality information and a rapid result is desirable to understand quality control effectiveness.

Purpose: This study verified the quantitative recovery, reproducibility, uncertainty and statistical difference ($P<0.05$) if any, between the 3M Petrifilm Rapid Aerobic Count (3M RAC) (AOAC OMA 2015.13, 35°C/24 h) and the 3M Petrifilm Aerobic Count (3M AC) (AOAC OMA 990.12, 35°C/48 h).

Methods: Two naturally contaminated samples (raw beef and raw ground beef) were purchased from a commercial establishment. Twenty sub-samples for each of the matrices were evaluated in comparison to the reference method per ISO 16140-3:2003. Study variables included different times, working shifts, days (10), lots (media, diluents) and analysts.

Results: The recovery, reproducibility and uncertainty obtained with the 3M RAC for the raw beef samples were comparable to the reference method (3.59 log CFU/g; 0.033 and 0.066 log CFU/g vs. 3.63 log CFU/g; 0.016 and 0.032 log CFU/g, respectively). Similarly the recovery, reproducibility and uncertainty obtained with the 3M RAC for the raw ground beef samples were comparable to the reference method (4.53 log CFU/g; 0.012 and 0.024 log CFU/g vs. 4.63 log CFU/g; 0.010 and 0.019 log CFU/g, respectively). Bland-Altman evaluation showed Bias values very close to zero (raw ground beef -0.12 and raw beef -0.05), demonstrating concordance between the two methods. For both samples, 3M RAC showed statistical equivalence $P<0.05$ (raw beef $P=0.817$ and raw ground beef $P=0.394$).

Significance: The 3M RAC method enabled reliable and rapid quantitative detection of aerobic bacteria in beef samples. The quantitative recovery with the new method was found to be statistically comparable to reference methodology and can provide actionable results to the food industry in as little as 24 h.

P2-34 Development of a Two-stage Label-free Aptasensing Platform for Rapid Detection of *Cronobacter sakazakii* in Powdered Infant Formula

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❖ ***Developing Scientist Competitor***

Introduction: *Cronobacter sakazakii* constitutes one of the most life-threatening foodborne pathogens in neonates, and is largely attributed to its presence in powdered infant formula. Despite the high risk of infection of *Cronobacter* spp., including *C. sakazakii*, most studies on the development of a *Cronobacter*-specific biosensor have been performed using self-developed antibodies, because there is currently no commercial antibody available against this bacterium.

Purpose: In this study, a sensitive and convenient two-stage label-free aptasensing platform was developed for colorimetric detection of *C. sakazakii* in powdered infant formula.

Methods: We selected aptamers against live intact *C. sakazakii* cells using the whole-cell SELEX, and then established a two-stage aptasensing platform using aptasensors based on the unmodified selected aptamers and gold nanoparticles for specific detection of *C. sakazakii* in realistic samples. The analytical performance of the developed platform was evaluated with artificially inoculated powdered infant formula.

Results: Under the optimum conditions determined in the experiments, *C. sakazakii* in powdered infant formula could be detected with the naked eye within 30 min at a concentration as low as 7.1×10^3 CFU/ml, and the linear range was from 7.1×10^3 to 7.1×10^7 CFU/ml.

Significance: To our knowledge, this is the first report of an aptamer against *C. sakazakii*, and of a label-free aptasensor to detect foodborne pathogens in food items. Finally, the platform may be customized with target-specific aptamers to detect other bacteria of interest.

P2-35 Quantitative Comparison of Pathogen Enrichment Strategies: Toward the Harmonization of Methods for the Recovery of *Shigella* from Produce

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Introduction: In the absence of a growth medium selective for *Shigella* only, the ability of these pathogens to grow in the absence of oxygen has been the enrichment strategy used in most methods for their recovery from foods. Success is dependent on their ability to grow and successfully compete with the resident microflora.

Purpose: To compare the influence of aerobic and anaerobic environments, and the impact of various chemical oxygen scavengers, on the growth efficiency of *Shigella* in competition with the natural flora from various produce types

Methods: Resazurin was added to the growth medium to demonstrate anaerobic conditions. Ten freeze-stressed Gfp-expressing *Shigella* cells were inoculated into the normal flora of spinach, field-grown tomato, parsley, cilantro and lettuce, using five replicates plus one unspiked control sample per experiment. Numbers of fluorescent bacteria and total bacteria were obtained pre and post enrichment, from serial dilutions onto non-selective Tryptic Soy Agar, to calculate growth factors and enrichment factors for each enrichment condition tested.

Results: The current anaerobic ISO method for *Shigella* was the most efficient method to recover the pathogen, with several logs difference in both the growth and the enrichment factors, in competition with the spinach natural flora, compared to the less efficient aerobic US Food-Emergency-Response-Network *Shigella* method. From the various modifications performed, i.e., nitrogen flush, nitrogen bubbling, adding oxyrase or various oxygen chemical scavengers, only the addition of nitrate had a more consistent positive effect on the recovery of *Shigella* from produce, in anaerobic condition.

Significance: Here we present a way to quantify the growth of a foodborne pathogen of interest, in competition with background microflora, leading to optimization of enrichment conditions for successful detection and/or recovery. We will further explore nitrate respiration as a selective step to anaerobic enrichment of *Shigella*, using additional food types.

P2-36 Development of a Rapid Diagnostic, ANSR™ *Campylobacter,* for the Detection of *Campylobacter* spp.

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Poster

Introduction: ANSR™ *Campylobacter* spp. is a molecular diagnostic that provides rapid and accurate detection of *Campylobacter* spp. in food samples. Following single-step enrichment, the assay uses isothermal nucleic acid amplification to detect *Campylobacter* spp. through binding of a molecular beacon.

Purpose: The purpose of this study was to assess the limit of detection and inclusivity/exclusivity in the development of a new rapid diagnostic assay for the detection of *Campylobacter* spp. in food matrices.

Methods: A rapid isothermal nucleic acid amplification assay targeting a specific, conserved 16s rRNA sequence within *Campylobacter* spp. has been developed. A simple lysis reaction releases target rRNA. Fifty µl of the lysate is added to the lyophilized reagent. A reverse transcriptase converts rRNA to DNA with amplification occurring at a constant 56°C. After amplification, a molecular beacon probe is used to detect amplified product.

Results: The ANSR™ *Campylobacter* spp. assay was shown to be inclusive for *C. jejuni, C. coli,* and *C. lari,* and exclusive against other genera tested. In an enrichment study, 30 ml of poultry rinse BPW spiked with *C. jejuni* (*n*=4), ranging from 0.73-1.56 CFU/ml, were all able to be detected after 16 hour enrichment in 2X Bolton's broth incubated at 42°C. Naturally occurring *Campylobacter* in poultry rinses was detected in samples that were tested prior to incubation. Upon plating onto CCA, it was found that the numbers of *Campylobacter* in these samples ranged from 10-55 CFU/ml. Turkey swabs were tested according to the USDA-MLG. Three were negative on CCA and ANSR after 48 hours incubation, one was positive after 24 hours while another was positive after 36 hours, reaching titers of 4.3x10e^3 CFU/ml and 1.3x10^{3}CFU/mL, respectively.

Significance: The new isothermal amplification assay provides the user with a simple, fast and effective tool for the detection of *Campylobacter* spp. in food matrices.

P2-37 Improvement of Karmali Agar by Supplementation with Tazobactam for Detecting *Campylobacter* from Chicken Carcass Rinse

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❖ ***Developing Scientist Competitor***

Introduction: Although there have been many commercialized *Campylobacter*-selective agars such as Campy-cefex agar, modified cefoperazone charcoal deoxycholate agar, and Karmali agar, it has been reported that these cefoperazone-based media are unsuitable for isolation of *Campylobacter* from poultry samples because the cefoperazone cannot suppress competing flora such as extended-spectrum-β-lactamase (ESBL)-producing *E. coli* sufficiently

Purpose: We compared the performance of tazobactam-supplemented Karmali agar (T-Karmali agar) with normal Karmali agar during the isolation of *Campylobacter* from whole chicken carcass rinse.

Methods: All samples were rinsed with 400 ml of buffered peptone water. The 25 ml of subsamples were enriched with 2 x blood-free Bolton enrichment broth prior to incubation at 42°C for 48 h. Pre-enriched broths were streaked onto Karmali and T-Karmali agar. The presumptive colonies were finally confirmed by colony PCR.

Results: The isolation rate of T-Karmali agar was slightly higher ($P > 0.05$) than that of normal Karmali agar (T-Karmali, 16 of 120; Karmali, 10 of 120). However, the selectivity (T-Karmali, 25 out of 120; Karmali, 99 out of 120) and growth index (T-Karmali, 1.36; Karmali, 2.83) of the T-Karmali agar was significantly better ($P < 0.05$) than that of normal Karmali agar.

Significance: The T-Karmali agar showed more selectivity than normal Karmali agar for isolation of *Campylobacter*.

P2-38 Improvement of Polymyxin-Egg Yolk-Mannitol Bromothymol Blue Agar for the Enumeration and Isolation of *Bacillus cereus* in Various Foods

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Introduction: Polymyxin-egg yolk-mannitol-bromothymol blue (PEMBA) has been recommended for the enumeration and isolation of *B. cereus* by food authorities.

Purpose: We developed modified PEMBA by supplementing trimethoprim to PEMBA to improve the selectivity for *Bacillus cereus* in various foods.

Methods: Field samples including red pepper powder (*n*=10), fermented soybean paste (*n*=10), *Saengsik* (*n*=10)*, Sunsik* (*n*=10), and fruit juice (*n*=10) were used to evaluate the performance of the novel media compared to PEMBA.

Results: In red pepper powder and soybean paste, the number of *B. cereus* in mPEMBA were significantly higher than that in PEMBA indicating better recoverability ($P < 0.05$). In addition, mPEMBA provided better visual differentiation of *B. cereus* colony than PEMBA, which is attributable to reduced number of competing flora.

Significance: In conclusion, the addition of trimethoprim to PEMBA could generate a synergistic effect to improve selectivity, suggesting mPEMBA could be a great alternative to PEMBA in isolating and enumerating *B. cereus* in various foods.

P2-39 Combined Detection and Strain Typing of *Yersinia enterocolitica* Directly from Pork and Poultry Enrichments

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Introduction: *Yersinia enterocolitica* is responsible for an estimated 98,000 cases of foodborne illness per year in the U.S., causing both intestinal and extraintestinal disease. Its prevalence in retail pork and poultry, believed to the primary sources of these infections, ranges widely from 0 to 69%. Conventional screening of food samples for this pathogen is problematic, requiring enrichment for 48 h or more in specialized media followed by isolation and speciation. Furthermore, *Y. enterocolitica* strains vary in their virulence, with serogroups O:3, O:8, O:9, and O:5,27 (of >60 total) most commonly associated with human disease.

Purpose: There is considerable need for a *Y. enterocolitica* combined detection/typing system that provides expedient, epidemiologically useful information at moderate cost.

Methods: We recently developed a novel approach to characterizing *Salmonella*, STEC, and *Listeria* strains directly from poultry and beef enrichment cultures. This enrichment, amplification, and sequence-based typing (EAST) approach was extended to *Y. enterocolitica* by targeting two polymorphic tandem-repeat containing loci, YeMT1 and YeMT2.

Results: Sequence analyses of 130 NCBI database strains yielded Simpson's diversity indexes of 0.98 and 0.97, respectively, for these loci. Cluster analyses of these sequences further showed that YeMT typing was largely congruent with serotype, biotype, and phylogroup. Application of YeMT-EAST to *Y. enterocolitica*-inoculated ground pork enrichments prepared as per FSIS guidelines (48 h in ITC medium) demonstrated that 10^4 CFU/ml or less (after enrichment) were sufficient for detection and typing. Initial studies with uninoculated ground pork and turkey enrichments revealed contamination with strains that cluster with previously characterized human fecal isolates. YeMT-EAST was subsequently tested with uninoculated retail chicken parts using conventional enrichment conditions (24 h in BPW medium); surprisingly, 10 of 11 samples (91%) were positive. However, cluster analysis suggests that these contaminants represent less pathogenic strains.

Significance: YeMT-EAST represents a potentially important new tool for investigating *Y. enterocolitica* epidemiology.

P2-40 MALDI-TOF MS Biotyping in the Characterization of Antimicrobial-resistant *Enterococcus* spp. from Wildlife Associated with Concentrated Animal Feeding Operations

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Introduction: Antimicrobial resistant (AMR) bacteria represent one of the most serious threats to food safety and security. Wildlife serve as reservoirs and have the potential to disseminate AMR bacteria across agricultural landscapes. Thus, control strategies to limit problem wildlife interactions with livestock and produce require efficacious methods to identify and characterize AMR within wildlife-associated bacteria.

Purpose: We aimed to develop matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the discovery of AMR-specific biomarkers and biosignatures. Our analyses were applied to species-level identification and characterization of AMR *Enterococcus* spp. collected from the gastrointestinal tracts of European starlings (*Sturnus vulgaris*), an invasive avian pest of concentrated animal feeding operations (CAFOs).

Methods: MALDI Biotyping was performed on 718 presumptive *Enterococcus* spp. isolates using the Bruker microflex LRF mass spectrometer operating with MALDI Biotyper RTC software (Version 3.4), following an ethanol/formic acid extraction of the bacteria. Antimicrobial susceptibilities of these isolates to 13 different antibiotics were then determined via disk diffusion assays. A machine learning approach of data interpretation was then applied to correlate the isolates' mass spectral signatures with antimicrobial susceptibility fingerprints.

Results: MALDI Biotyping confidently identified (Biotyping score ≥ 1.90) 658 of the 718 isolates as *Enterococcus* spp. (91.6%), consisting of *E. casseliflavus*, *E. durans*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. hirae*, *E. mundtii*, and *E. villorum*. Identification of 14 isolates was not possible (1.9%), and the remaining isolates were of the genera *Aerococcus*, *Staphylococcus*, and *Vagococcus*. Importantly, the application of the machine learning strategy enabled the identification of multiple ions and ion pairs that were predictive of specific antimicrobial susceptibility phenotypes.

Significance: This study demonstrates that MALDI-TOF MS is a feasible strategy for the identification of wildlife-associated *Enterococcus* spp. and for discriminating between AMR phenotypes.

P2-41 Comparison of Detection Methods for *Bacillus anthracis* in High Background Food Matrices

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Introduction: *Bacillus anthracis*, the etiological agent of anthrax, can cause severe zoonotic disease in humans. While strong cultural detection methods exist, a reliable screening method is imperative for ruling out samples in large volumes of food samples. This study is based upon a parallel comparison between four commercially available *B. anthracis* detection kits and four culture media in pre and post enriched ground in beef.

Purpose: This study's objective was to conduct a comparison study between commercially available kits and culture media for the detection of *B. anthracis* in high background food matrices.

Methods: Matrix limit of detection studies were conducted in parallel on the Smart II, BADD, Biothreat Alert, and Tetracore kits and select cultural media from both direct and overnight enrichments. Four fortification levels of anthrax were fortified in ground beef ranging from 1x10^3 to 1x10^6 CFU/g. The manufacturer's instructions were carried out for each method. Each kit method and fortification level was replicated in triplicate. The ELISA method was read at the endpoint absorbance at 405 nm and cultural plates were enumerated after overnight incubation. Non-target strains were examined for exclusivity.

Results: The kits evaluated post enrichment, the lateral flow device BADD performed with a 66.7% detection sensitivity at 1x10^4 CFU/g. Tetracore ELISA kits demonstrated a 66.7% sensitivity at 1x10^4 CFU/g, but a 100% sensitivity at 1x10^5 CFU/g post enrichment. All kits demonstrated 100% specificity against exclusivity strains. SBA, MYP, ACA, and PLET demonstrated high levels of *B. anthracis* and non-target bacteria post enrichment.

Significance: The data from this study suggest food matrices contaminated with >1x10^4 CFU/g, the BADD LFD kits and Tetracore ELISA kits may offer a suitable screening tool for the detection of *B. anthracis* in foods, post enrichment.

P2-42 Assessment of the BP+ Agar for the Enumeration of *S. aureus* in Cheeses with Edible Rind

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Introduction: When analyzing cheese samples for the enumeration of *Staphylococcus aureus*, the edible rind is not typically included in the analysis as it contains significant microflora capable of forming a lawn of growth on Baird Parker agar (BP). This practice makes it impossible to obtain an accurate quantitative assessment of *S. aureus* if present in the sample. One solution to obtain isolated colonies is to further dilute the analytical sample; however, by diluting the competitor flora, the concentration of *S. aureus* is also diluted and the probability of detecting this microorganism decreases.

Purpose: BP+ agar has already demonstrated its equivalence to BP agar for the enumeration of *S. aureus* in raw milk cheese without the rind while significantly inhibiting background microflora. In this project, BP+'s effectiveness for the enumeration of *S. aureus* in raw milk cheese with the rind was assessed.

Methods: The laboratory analyzed more than 100 cheeses with edible rind using Health Canada's MFHPB-21 method - Enumeration of *Staphylococcus aureus* in food. BP and BP+ agars were plated in parallel for the analysis of cheese sample, which were each analyzed with and without the rind. The evaluation criteria for validation were 1) equivalence of *S. aureus* counts, when the microorganism is present, 2) inhibition of the cheese microflora, 3) number of downstream analytical confirmations that could be avoided by using the BP+.

Results: In 10% of the analyzed samples, *S. aureus* was detected only in those containing the cheese's rind. This observation supports the need to include the rind in the analytical portion to adequately evaluate the risk to consumers.

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Significance: Given the observed performance of BP+, its use is an added value for the analysis of a representative portion of edible rind cheeses. Food microbiology laboratories will benefit from a method capable of analyzing the higher risk portions of these types of cheese, thus increasing the probability of detecting *S. aureus*.

P2-43 Droplet Digital PCR Method for Multiple Gene Marker Determination in Single Cells Enabling Accurate Detection of Priority STEC in Food Enrichment Cultures

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❖ ***Developing Scientist Competitor***

Introduction: The identification of priority Shiga toxin-producing *E. coli* (STEC) requires detection of characteristic gene markers (i.e., Shiga toxin (*stx*) and intimin (*eae*)). Microbiological methods for detection of STEC in foods often use PCR-based approaches to screen enrichment broths for these markers. However, false positives arise when complex food matrices such as beef contain both *eae*-negative STEC and *eae*-positive *E. coli*, and no STEC with both markers in a single cell.

Purpose: To reduce false positive detection of priority STEC, we have developed a multiplexed droplet digital PCR (ddPCR) assay capable of detecting *stx* and *eae* genes in a single bacterial cell.

Methods: A novel method involving: (1) dispersal of intact bacteria into droplets; (2) release of gDNA by heat lysis; and (3) amplification and detection of genetic targets (*stx* and *eae*) using standard TaqMan chemistries and the BioRad QX200™ ddPCR system was tested with panels of target STEC and non-target *E. coli*.

Results: By determining the proportion of droplets positive for both targets relative to all positive droplets, samples containing priority STEC (typically 20-40% double-positive) could be distinguished from samples containing mixtures of *eae*-negative STEC and *eae*-positive *E. coli* (0-2% double-positive). The use of intact cells was necessary as this linkage was not observed with gDNA extracts. In simulated enrichment broths, STEC could be accurately identified in backgrounds with up to 1,000 x generic *E. coli* relative to target organisms. Similar sensitivity was achieved in spiked enrichment broths from ground beef and produce samples.

Significance: To our knowledge, this is the first report of dual-target detection in single bacterial cells using ddPCR. The application of this assay to enrichment-culture screening would reduce false-positives, thereby improving the cost, speed and accuracy of current methods for STEC detection in foods.

P2-44 Selection of Aptamers Using Whole-Bacterium SELEX for Rapid Detection of *E. coli* O157:H7

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❖ ***Developing Scientist Competitor***

Introduction: *E. coli* O157:H7 is one of the top five pathogens contributing to foodborne diseases, causing an estimated 2,138 cases of hospitalization in the US each year. The extremely low infectious dose demands for more rapid and sensitive methods for detecting *E. coli* O157:H7.

Purpose: The objective of this study is to use whole-bacterium SELEX (Systematic Evolution of Ligands by Exponential Enrichment) for the selection of aptamers specifically binding to *E. coli* O157:H7, which could later be used for the development of aptamer-based biosensors for rapid detection of target bacteria.

Methods: The whole-bacterium SELEX procedure included four steps: the binding of aptamer candidates to *E. coli* O157:H7 cells captured by immunomagnetic beads, the partitioning of bound aptamers from unbound DNA oligonucleotides by magnetic separation, the amplification of bound aptamers with PCR, and finally the regeneration of aptamers as the input for the next cycle. Following all cycles of SELEX, the aptamer pool with the highest binding affinity was cloned and sequenced. The binding affinity of the synthesized aptamer was evaluated by dot ELISA.

Results: A total of 19 cycles of selection and 6 cycles of counter-selection were performed. In counter selection, the aptamers were selected against a mixture of *S. aureus*, *L. monocytogenes* and *S.* Typhimurium. Among twenty colonies that were sequenced, only 3 different sequences were obtained, one of which repeated 16 times and was then synthesized. The results of dot ELISA showed that as the concentration of aptamers increased from 10 nM to 1,000 nM, the binding affinity to *E. coli* O157:H7 increased.

Significance: Ongoing researches focus on the kinetic analysis of selected aptamer and the comparison between the aptamer and the anti-*E. coli* antibody in both sensitivity and specificity. The selected aptamer may become a substitute of antibodies in the development of biosensors for rapid detection of *E. coli* O157:H7.

P2-45 An Independent Laboratory Evaluation of the Mericon *E. coli* Detection Workflows for AOAC-RI PTM Status

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Introduction: Regulation of pathogenic *E. coli* in foods is receiving heightened attention under new food safety requirements. The *mericon E. coli* O157 Screen Plus and *E. coli* STEC O-type detection methods combine straightforward sample prep with real-time *PCR* detection. A choice of two different DNA prep procedures can be utilized; the manual *mericon* DNA Bacteria Kit or the automated QIAsymphony *mericon Bacteria Kit*. The resultant purified DNA is combined with *mericon* PCR Master Mix and real-time PCR is conducted on the Rotor-Gene Q platform.

Purpose: To conduct a method comparison, inclusivity/exclusivity and ruggedness studies on the *mericon E. coli* kits as part of the AOAC Research Institute PTM process.

Methods: For the method comparison, 3 foods were analyzed (raw ground beef (70% lean), beef trim, and fresh spinach). Each matrix was inoculated with a different serotype of pathogenic *E. coli* at 2 levels (0.2-2 CFU/test portion and 2-5 CFU/test portion) and cultured for 10 +/- 1 h in mTSB at 41°C. For each sample, DNA was extracted by both methods, analyzed by both *mericon* real-time PCR assays and compared to the MLG or BAM reference methods. Test kits were also evaluated for ruggedness, inclusivity and exclusivity.

Results: For inclusivity, all 50 strains were positively detected. For exclusivity, all 30 strains were correctly excluded. The ruggedness study indicated that minor changes to the DNA extraction and PCR analysis did not affect the outcome. The method comparison demonstrated no significant differences between the number of positive samples detected by the *mericon* and reference methods for all 3 matrices. The *mericon E. coli* methods were granted AOAC RI PTM status.

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Significance: This new method is an efficient, rapid and reliable alternative to the traditional methods of detecting *E. coli* virulence factors and Big 7 STEC O-type in several foods.

P2-46 A Nanowell-based Immunosensor for Rapid and Sensitive Detection of *E. coli* O157:H7

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Introduction: *Escherichia coli* O157:H7 is one of the most dangerous foodborne pathogens. The development of a rapid, sensitive, and specific detection method for *E. coli* O157:H7 is continuously needed to improve food safety and security.

Purpose: The objective of the present study was to develop a nanowell-based immunosensor using a screen printed interdigitated electrode (SPIE) for rapid and sensitive detection of *E. coli* O157:H7 in foods.

Methods: A nanoporous-gold-film (NGF) with a thickness of 120 nm and a pore size of ~20 nm was prepared by a metallic corrosion method. Then, the NGF was coated onto the gold surface of a SPIE through a self-assembled monolayer to form a nanowell-based electrode. Polyclonal antibodies against *E. coli* O157:H7 were immobilized on the electrode surface through covalent bonding. After a blocking solution was applied, a test sample was added. Target *E. coli* O157:H7 presented in the sample was captured by the immobilized antibody, resulting in a change in the impedance of the nanowell-based electrode surface.

Results: The results showed that the developed nanowell-based immunosensor was able to detect *E. coli* O157:H7 with an enhanced impedance signal when compared to an immunosensor without nanowell. The impedance value of the nanowell-based electrode was observed to increase a 10-fold for detection of *E. coli* O157:H7 in pure culture at a concentration of 10^5 CFU/ml in an applied volume of 45 µl. It was a label-free detection with a total detection time from adding the sample to obtain the final result within one hour. Further work will be concentrated on the optimization of conditions of the nanowell-based immunosensor, and detection of target bacteria in different foods.

Significance: The outcome of this study will improve the sensitivity of the immunosensor due to the greatly increased gold surface area with the help of nanowell-based electrodes, which will provide a sensitive, rapid and label-free method for detection of foodborne pathogens.

P2-47 A Hand-held Electrochemical Biosensor with Glucose Oxidase-polydopamine Based Polymeric Nanocomposites and Prussian Blue Modified Screen-printed Interdigitated Microelectrodes for the Detection of *E. coli* O157:H7 in Foods

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❖ ***Developing Scientist Competitor***

Introduction: The possible presence of pathogenic bacteria in foods has always been a great threat to the wellbeing of people and the revenue of food manufactures. Therefore, the demanding for advanced detection methods that can rapidly and sensitively detect these pathogens using a portable device for on-line or in-field applications has been of great importance.

Purpose: In this study, a handheld electrochemical biosensor for the detection of *E. coli* O157:H7 was developed with the integration of glucose oxidase (GOx)-polydopamine (PDA) based polymeric nanocomposites (PMNCs) for carrying and labeling the target bacteria and Prussian blue (PB) modified screen-printed interdigitated microelectrodes (SP-IDMEs) for electrochemical measurement.

Methods: The PMNCs consisting of MBs and abundant GOx in the matrix of PDA were first synthesized by the self-polymerization of dopamine (DA) under alkaline condition. Gold nanoparticles (AuNPs) were immobilized on the surface of PDA-GOx-MBs PMNCs through biochemical synthesis to achieve further high efficient adsorption of antibodies (Abs) and GOx. The final product of Abs/GOx_{out}/GOx-MBs@PDA PMNCs was used to capture target bacteria and separate them from food matrix. The PMNCs-cells conjugates were then filtered through a filter paper, and the free PMNCs were collected after filtration. The collected PMNCs were transferred into glucose solution to allow the enzymatic reaction. The amperometric detection can be measured with a handheld electrochemical detector using PB-modified SP-IDME.

Results: The constructed biosensor had been proved to be able to detect *E. coli* O157:H7 in pure culture and the detection limit was achieved about10^2 CFU/ml. Ongoing research has focused on the validation of the biosensor with food sample like ground beef.

Significance: The developed biosensor exhibited high load of enzyme through PMNCs for efficient amplification of signals and applied a portable device for easy and rapid measurement in detection of foodborne pathogens.

P2-48 Sensitive Detection of *Escherichia coli* O157:H7 Based on Cascade Signal Amplification in ELISA

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Introduction: Foodborne pathogens are a major public health issue worldwide. Thus, a sensitive method for pathogen detection should be developed to achieve food quality and safety.

Purpose: In this study, cascade signal amplification in ELISA involving ds-ELISA and ic-ELISA was established to sensitively detect *Escherichia coli* O157:H7.

Methods: A complex was formed comprising anti-*E. coli* O157:H7 pAb, *E. coli* O157:H7, biotinylated anti-*E. coli* O157:H7 mAb, streptavidin, and biotinylated β-lactamase in ds-ELISA. Penicillin solution was then added into the ELISA well and hydrolyzed by β-lactamase. Afterward, the penicillin solution was transferred to ic-ELISA and sensitively detected.

Results: In the cascade signal amplification system, increasing the amount of added *E. coli* O157:H7 resulted in more β-lactamase and less penicillin. The detection sensitivity of *E. coli* O157:H7, which was 20 CFU/ml with the cascade signal amplification in ELISA, was 1,000 higher than that of traditional ELISA. Furthermore, the novel method can be used to detect *E. coli* O157:H7 in milk.

Significance: This new signaling strategy will facilitate analyses of highly sensitive foodborne pathogens.

P2-49 Evaluation of GFP Reporter-labeled Control Strains for Shiga Toxin-producing *Escherichia coli* (STEC) Assays

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Poster

Introduction: Growing concern over bacterial food contamination has led to increased examination of food testing protocols in today's industry. Currently, the use of bacterial strains as positive controls in testing protocols is not widely practiced for fear of cross-contaminating samples. Due to ongoing scrutiny of food testing methodology and growing regulations under the Food and Drug Administration (FDA) Food Safety Modernization Act, it is imperative to have control strains with unique, easily detectable traits that distinguish positive control strains from actual food contaminants, diminishing the fear of cross-contamination and improving current practices. We have created green fluorescent protein (GFP) reporter-labeled *Escherichia coli*strains, including Shiga toxin-producing O157, for use as controls in QC testing.

Purpose: To evaluate the use of *E. coli*labeled with GFP as a positive control in media and food safety testing.

Methods: A plasmid encoding the synthetic Dasher GFP (DNA 2.0) was transformed into toxigenic *E. coli* O157:H7 and different serogroups of the "Big Six" non-O157 *E. coli* (ATCC® MP-9™) strains. The stability of the reporter was determined through unselective serial passage and by plating on tryptic soy agar, following the USDA FSIS MLG Chapter 5.05 protocol. Additionally, the growth rate and chromogenic properties of reporter-labeled and native strains were compared.

Results: The GFP-transformed STEC colonies were readily detectable with the naked eye when exposed to a 310 nm UV light source. Reporter-labeled strains containing GFP were compared with their native strains to identify phenotypic changes on Rainbow Agar™ (Biolog), a chromogenic media commonly used to assist in the identification of *E. coli* serotypes. Minimal color variations between the reporter versus native strains were observed. To determine the effect of GFP expression on the growth and viability of the labeled *E. coli*, growth studies were performed using BioScreen C™ (Growth Curves, USA). Minimal optical density variation was detected. To determine GFP reporter stability in these engineered strains, they were passaged once every 24 hours under temperature stress at 42°C. The percentage of GFP positive colonies was 60-100% after two days (percentage variation was strain dependent), permitting their use as controls in the intended qualitative food testing workflows.

Significance: This study demonstrates that these GFP-labeled *E. coli* strains can be routinely used as positive controls for media testing and in the detection of microbial pathogens in food.

P2-50 Performance of a New Molecular Method for the Detection of *E. coli* O157

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Introduction: Improved rapid methods for foodborne pathogen detection are an ongoing need. A new molecular method for the detection of *E. coli* O157 has been developed based on modification of an existing product.

Purpose: To evaluate the performance of a new *E. coli* O157 isothermal molecular detection method for 1) inclusivity; 2) exclusivity; and 3) comparison to the ISO cultural method.

Methods: Inclusivity evaluation was performed using 60 different *E. coli* O157 cultures and exclusivity using 60 different non-*E. coli* O157 cultures. True positive and true negative states were defined by each organism's genetic or biochemical identity. A method comparison was performed by testing 60 produce samples including various sprouts, fresh leafy greens, cilantro and parsley. Samples were artificially contaminated at a low level or left non-inoculated, then were acclimated before testing. Non-paired matrix samples were analyzed using the ISO 16654 reference method and the new isothermal molecular method after incubation in Buffered Peptone Water-ISO at 41.5°C for 18 hours. All results were confirmed using the ISO method protocol.

Results: Inclusivity and exclusivity of 100% were determined using pure cultures. For the method comparison, there was positive agreement among 28 samples, negative agreement among 31 samples and one negative deviation. According to ISO 16140-2:2015, this result meets the acceptability limit criterion for alternative method performance, demonstrating the capability of the new isothermal molecular method for detection of *E. coli* O157 in these matrices.

Significance: The new isothermal molecular detection method was evaluated using cultures of known identity, and sample matrices were tested in comparison to a reference cultural method. The new method was determined to be reliable and accurate and to offer substantial advantages to the end user, including a quicker time to result compared to the cultural method.

P2-51 A Novel Phage-based *Escherichia coli* O157:H7 Detection Method for Ground Beef

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Introduction: Existing protocols for detection of *E. coli* O157:H7 in foods are complicated, expensive, slow, labor-intensive and prone to false positives. The PhageDx *E. coli*O157:H7 Assay was developed to address most of these shortcomings, offering beef producers an effective, fast and simple testing alternative.

Purpose: To assess the feasibility of PhageDx *E. coli* O157:H7 Assay to detect at least one CFU of *E. coli* O157:H7 in 25 g ground beef (GB) in less than 7 h, from sample preparation to results.

Methods: GB samples (25 g) were inoculated with *E. coli* O157:H7 and stored for 24 h. GB was mixed at 1:3 with tryptic soy broth, homogenized, incubated for 5 h, centrifuged (10 ml aliquot), and infected with a luciferase-expressing recombinant *E. coli* O157:H7 phage for 2 h. After substrate addition, samples were read directly in a luminometer.

Results: A single CFU of *E. coli* O157:H7 was detected in GB samples after 7 h in 21 separate samples. All 58 *E. coli* O157:H7 strains yielded a strong positive signal when tested in pure culture. None of 132 other bacteria, including 12 non-O157:H7 STEC strains, 120 commensal *E. coli*, and other coliform, *Listeria, Salmonella,* and *Staphylococcus* species, yielded positive luminometer signals when tested in pure culture.

Significance: The PhageDx *E. coli* O157:H7 Assay offers a valuable testing alternative for beef producers compared to the most commonly used protocols. Benefits include a time-to-results (TTR) reduction of approximately 40% to 50% to release inventory faster, a simple five-step protocol, and fewer false positives.

P2-52 A Unique Phage-linked Approach to Detect *Escherichia coli* O157:H7 in Water Samples

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Introduction: The detection of *E. coli* O157:H7 in water samples is a new challenge for the beverage and produce industry, and few commercial protocols have been developed for this purpose. Traditional methods typically require overnight incubation and may lack sensitivity.

Purpose: To assess the feasibility of PhageDx *E. coli* O157:H7 Assay to detect fewer than three CFU *E. coli* O157:H7 in 100 ml water in 4 h, from sample preparation to results.

Methods: Water samples (100 ml) were inoculated with known numbers of *E. coli* O157:H7, filtered onto PVDF membranes, enriched for 2 h, and infected with a luciferase-expressing recombinant *E. coli* O157:H7 phage for 2 h. Enrichment and infection steps were performed directly on the filter membrane. One-hundred microliters of samples were transferred to a 96-well plate, substrate was added, and signals were measured in a standard luminometer.

Results: A single CFU of *E. coli* O157:H7 was detected in 100 ml room-temperature water samples in 4 h with signals ranging from 3- to 6-fold above background. Host-range studies of the *E. coli* O157:H7 phage indicates it is highly specific for *E. coli* O157:H7 as positive luciferase signal are seen with 58 *E. coli* O157:H7 strains, while little or no signal was observed with 132 other bacterial strains, including commensal *E. coli*, 12 non-O157:H7 Big 6 STEC, and other coliforms.

Significance: Current water testing methods are characterized by 12 - 24 h time-to-results, visual identification of samples, and the possibility of missing potentially contaminated samples. The option to test water samples quickly and accurately would benefit irrigation and drinking water testers, as well as beverage and vegetable producers.

P2-53 Concentration of *Escherichia coli* O157:H7 from Experimentally Infected, Pre-packaged Spinach by InnovaPrep's Concentrating Pipette

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Introduction: Many food outbreaks due to *Escherichia coli* O157:H7 are associated with vegetables and fruits. Because these are perishable food items, procedures enabling same-day detection of low levels of pathogens are needed. In this study, InnovaPrep's Concentrating Pipette was investigated as a bridge to concentrate large sample sizes into volumes more appropriate for rapid detection, allowing for reduced pre-enrichment times.

Purpose: The purpose of this study was to determine if same-day detection of experimentally infected spinach for *E. coli* O157:H7 is achievable.

Methods: An average of 10 CFU of *E. coli* O157:H7 was inoculated into 25 grams of pre-packaged fresh spinach, which was homogenized by a stomacher (AES) in 225 ml pre-warmed tryptic soy broth in a stomacher bag containing a 250 micron pre-filter (Interscience). After 5 ½ hours of incubation, 100 ml of the homogenate was concentrated with a single-use, 0.45µm hollow fiber Concentrating Pipette Tip. The eluted samples where then subjected to qPCR using DuPont's BAX system and additionally plated onto tryptic soy agar plates.

Results: Each experimentally infected spinach sample that was subjected to qPCR resulted in a positive detection (*n*=9), whereas blank controls were negative (n=3). For concentration, the average time to concentrate 100 ml of homogenized spinach samples was 3.79 minutes ± 2.89. The average concentration elution volumes were 216.70 µl ± 34.17 µl, which resulted in an average concentration factor of 349.18X ± 186.87X. Average time-to-result was 8 hours and 20 minutes.

Significance: These data suggest that spinach testing for *E. coli* O157:H7 can be performed in nearly the same time as an 8-hour work shift.

P2-54 Validation of Test Portion Pooling for the Detection of *Listeria* spp. and *L. monocytogenes* in Dairy Products

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Introduction: *Listeria monocytogenes* is a major foodborne pathogen. Testing of multiple portions of a same food item is often required to verify the effectiveness of a HACCP plan. It will be advantageous to the laboratories to combine these test portions and process as one sample as it will result in efficiency.

Purpose: Validate pooling of test portions for the detection of *L. monocytogenes* and *Listeria* spp. in dairy products as no scientific evidence currently exists to support this practice.

Methods: Six matrices, namely, pudding, yogurt, brie cheese, 2% milk, ice cream and infant formula were spiked separately with stressed *L. monocytogenes* and *Listeria* spp. in 25 g and pooled test portions (375 g/ 250 g/125 g). ISO 11290-1:1996 Amd1:2004 and Rapid L'mono methods were followed until confirmation step for the detection of the spiked organisms. Performance of a method in pooled test portions was considered to be satisfactory if the relative limit of detection ($RLOD_{50}$; LOD_{50} [pooled test portion] / LOD_{50} [25-g test portion]) obtained was ≤ 2.5 (acceptance criteria following draft ISO 16140-2).

Results: Acceptable $RLOD_{50}$ (≤ 2.5) values were consistently obtained when test portions were pooled up to 125 g for all matrices tested. Obtained $RLOD_{50}$ values ranged from 0.719 to 1.666 and 0.568 to 2.435 for the detection of *L. monocytogenes* and *Listeria* spp., respectively, using both methods. LOD_{50} values obtained from the testing of 125-g test portions was approximately 1 CFU for most of the matrices. Though satisfactory $RLOD_{50}$ values were obtained for milk (0.568 – 1.132) and infant formula (0.874 – 1.666) matrices when pooled up to 375 g, it was not the case for other food items.

Significance: Pooling of test portions to up to 125 g for the detection of *L. monocytogenes* and *Listeria* spp. by two culture methods in dairy products has been validated.

P2-55 Detection of *Listeria* Species in Naturally and Artificially Contaminated Chicken Meat and Environment Samples: A Comparison of the Reference Method to 3M Molecular Detection Assay 2 Listeria

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Introduction: *Listeria* species are commonly isolated from food processing environments. Detection of *Listeria* species is typically used to verify sanitization programs and as an indicator for potential contamination by *Listeria monocytogenes.* Rapid and accurate detection of *Listeria* species can prevent introduction of contaminated foods into the markets and recalls.

Purpose: This study aimed to evaluate the 3M Molecular Detection Assay 2 *Listeria* (3M MDA 2 *Listeria*) compared to the conventional ISO 11290-1 method.

Methods: A total of 109 chicken meat samples and 110 environmental samples were screened in duplicate for *Listeria* using ISO 11290-1 culture method and 3M MDA 2 Listeria. *Listeria innocua* and *Listeria ivanovii* (at 200, 20, 2, 0.2, 0.02, and 0.002 CFU per unit) were used to artificially inoculate samples (*n* = 49 chicken meat and *n* = 50 environmental samples) to determine accuracy, sensitivity, and specificity. PCR targeting *prsA* gene and sequencing were performed on presumptive colonies to confirm the genus.

Results: Of the naturally contaminated chicken meat (*n* = 60) and environmental (*n* = 60) samples, 21 and 20 samples were positive for *Listeria* by the conventional method and the 3M MDA 2 *Listeria*, respectively. The 3M MDA 2 detected *Listeria* species with 92.66% relative accuracy, 93.65% relative specificity, and 91.30% relative sensitivity from 109 chicken meat samples, naturally and artificially contaminated. From 110 environmental

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samples the percentages for relative accuracy, specificity, and sensitivity were 91.82, 91.04, and 93.02, respectively. The detection limit for *Listeria* spp. was < 2 CFU per unit.

Significance: This study demonstrated that for the 3M MDA 2 *Listeria* detection of this microorganism is reliable and accurate compared to ISO 11290-1. This rapid molecular technique offers a faster response time to control contamination in the food production chain.

P2-56 Parallel Study Comparing Conventional ISO 11290-1 and 3M Molecular Detection Assay 2 *Listeria monocytogenes* for the Detection of *Listeria monocytogenes* in Chicken Meat and Its Processing Plant Environment, in Thailand

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Introduction: *Listeria monocytogenes* is a foodborne bacterial pathogen associated with life-threatening invasive diseases in humans and animals. It is also among the top causes of food recalls worldwide. *Listeria monocytogenes* is a threat to both public health and food industry. Early detection of *Listeria monocytogenes* contamination can save lives and prevent recalls.

Purpose: This study aimed to evaluate the 3M Molecular Detection Assay 2 *Listeria monocytogenes* (3M MDA 2 *Listeria monocytogenes)* in comparison to the conventional ISO 11290-1 method.

Methods: Sixty 25-gram chicken meat samples and 60 environmental samples (sponge sticks) were collected from chicken processing plants. Additionally (*n*=49) chicken meat and (*n*=50) sponge with letheen broth samples were artificially inoculated with *Listeria monocytogenes* using 200, 20, 2, 0.2, and 0.02 CFU/sample to determine the each method's sensitivity. Duplicate sets of each sample were screened for *Listeria monocytogenes* using ISO 11290-1 and 3M MDA 2 *Listeria monocytogenes*. PCR targeting the genus specific *prsA* and species specific *hly* genes and sequencing were used on presumptives to confirm genus and species.

Results: Of the 120 naturally contaminated samples, 6 and 7 samples were positive for *Listeria monocytogenes* by ISO 11290-1 and 3M MDA 2 *Listeria monocytogenes*, respectively. Of the 109 chicken meat samples, 3M MDA 2 *Listeria monocytogenes* detected *Listeria monocytogenes* with 96.33% relative accuracy, 98.85% relative specificity, and 86.36% relative sensitivity on naturally and artificially contaminated samples. In the 110 sponge samples, relative accuracy, specificity, and sensitivity were 97.27%, 96.97%, and 100%, respectively. The detection limit was < 2 CFU.

Significance: This study demonstrates that compared to ISO 11290-1, the 3M MDA 2 *Listeria monocytogenes* is reliable and accurate and at the same time this rapid molecular detection assay offers a faster time to result in the detection of *Listeria monocytogenes* in food processing.

P2-57 Detection of *Listeria monocytogenes* in Soft Cheese Using a Shotgun Metagenomics

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Introduction: *Listeria monocytogenes* is a major foodborne pathogen present in a variety of foods such as soft cheese made with pasteurized milk. The microbe causes invasive syndromes with case fatality rates as high as 30% in specific populations such as the elderly, immune-compromised populations. Metagenomics can be applied to develop a faster and more robust method to analyze milk-based products for bacterial pathogens such as *Listeria monocytogenes.*

Purpose: The objective of this study was to assess the efficacy of a metagenomics approach for strain-level detection of *L. monocytogenes* in soft cheese in both pre-enriched and subsequent enriched samples.

Methods: Soft cheese samples were spiked with *L. monocytogenes* and were processed according to the FDA BAM method and prepared for sequencing libraries. Sequencing data was analyzed using a k-mer signature method, an in-house analytical tool, to identify indigenous microbial populations. In addition, *L. monocytogenes*-specific DNA fragments were assembled and used to confirm the identity of the contaminating pathogen.

Results: The indigenous bacteria in soft cheeses were analyzed at several time points during pre-enrichment and enrichment for both spiked and unspiked samples. Our metagenomic analysis identified *L. monocytogenes* specific molecular markers and enabled assembly of the *L. monocytogenes* genome at level as low as 1,000 CFU/g soft cheese without requiring overnight culture.

Significance: A metagenomics approach may provide a faster and more rapid method to detect and identify *L. monocytogenes* contamination in soft cheeses as compared to conventional culture methods, and thus, improving food safety and outbreak response efficiency.

P2-58 Detection of *Listeria monocytogenes* Using a Liquid Crystal-based Immunoassay

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Introduction: Patients infected with *Listeria monocytogenes (L. monocytogenes*) exhibit the highest mortality rate experienced with foodborne bacterial pathogens. *L. monocytogenes* is additionally challenging because of its long persistence at low temperatures on surfaces and niches in food processing plants. The current study developed a rapid (< 30 hours sampling to results), one step enrichment, liquid crystal (LC)-based screening test for detection of *L. monocytogenes* on surfaces.

Purpose: The LC assay was developed, tested for selectivity and specificity, and compared against two culture agars and the USDA reference method.

Methods: The LC assay selectivity and specificity was evaluated using four enrichment media. Surface testing on stainless steel and plastic was conducted 24 hours after the sample was inoculated and dried at room temperature. Swab samples were collected, enriched and tested. LC assay detection was compared against direct plating on Modified Oxford Agar (MOX) and Chromagar Listeria, and the USDA reference method.

Results: Foodchek Listeria and University of Vermont (UVM) media provided superior growth and selectivity when combined with the LC assay. The LC assay detected 98% (54 of 55) of the *L. monocytogenes* and excluded 95% (38 of 40) of the non-*L. monocytogenes* tested. Detection by the LC assay was not statistically different (*n*=84, $dPOD_c$= 0.05 with a 95% confidence interval of (-0.10, 0.19)) when compared to MOX and Chromagar Listeria, and was not significantly different from the USDA reference method.

Significance: The liquid crystal-based immunoassay provides a rapid, one-step enrichment screening method for surface-associated *L. monocytogenes*.

P2-59 Fast Detection of *Listeria monocytogenes* in Deli Meat and Dairy Products

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Introduction: The ability of *L. monocytogenes* to proliferate in various foods at refrigeration temperatures and survive even after deep freezing makes the occurrence of this foodborne pathogen in ready-to-eat (RTE) foods of particular concern. It is especially threatening to the deli meat and dairy industries if fast and reliable detection methods are not applied. Since *L. monocytogenes* in RTE food can be present at low concentration with sub-lethal injury during food processing, an enrichment step is crucial to resuscitate injured cells and allow sufficient growth for detection.

Purpose: The objective of this study was to validate a sensitive and rapid method for *L. monocytogenes* detection in deli meat and dairy products.

Methods: Deli meat (cold smoked turkey and cured ham) and dairy product (double chocolate ice cream and pasteurized milk) samples were artificially contaminated with sub-lethally heat-stressed *L. monocytogenes* of different serotypes and stabilized for 48–72 hours at 2–8°C or for 14 days frozen. Samples were enriched in Actero™ Listeria Enrichment Media, then processed with the DuPont™ BAX® System Real-Time PCR Assay for *L. monocytogenes*.

Results: A total of 240 artificially contaminated food samples were examined to evaluate performance of the candidate method in comparison with the appropriate USDA-FSIS or US FDA reference method. Additionally, efficacy of the method was evaluated with four other ice cream flavors (vanilla, strawberry, caramel with pecan and cookie dough) as well as with 375 g samples of double chocolate ice cream. Optimization studies resulted in significant reduction in the enrichment phase with incubations of 18-22 hours for dairy products and 24 hours for deli meats. Method comparison studies demonstrated no false positive results or false negative results with the candidate method.

Significance: According to the AOAC International Probability of Detection statistical model, the candidate method was equivalent to the reference methods.

P2-60 Evaluation of DNA Extraction and Real-time PCR Screening Method for *Listeria monocytogenes* and *Listeria* spp. from Cantaloupe Peel and Queso Fresco Cheese

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Introduction: *Listeria monocytogenes* is a significant foodborne pathogen. Detection traditionally includes enrichment with isolation on selective media. Real-time PCR (qPCR) screening would assist rapid detection in food samples. Optimal DNA template preparation removes PCR inhibitors, concentrates target and improves overall qPCR sensitivity.

Purpose: Evaluate semi-automated DNA extraction with qPCR detection for *L. monocytogenes* and *Listeria* species from cantaloupe peel and soft cheese.

Methods: Two runs per matrix included 20 low (≈0.5 CFU/g), 5 high (≈5 CFU/g) *L. monocytogenes* inoculum levels and 5 uninoculated sample replicates. Surface inoculated cantaloupes were air dried and aged 48 h. Cheese samples were inoculated and aged 72 h at 4°C. Samples analyzed per BAM. Also, 48-h enrichments screened by preparing DNA template on MagMax Express 96 and analyzed by LIS-7500 multiplex qPCR assay on AB 7500 platform. Three replicate qPCR tests were run from each DNA template. The LIS-7500 assay targets *iap* gene in *L. monocytogenes* and *Listeria* spp. plus an internal amplification control.

Results: The qPCR screen detected *Listeria* in 15/20 and 14/20 at low, 4/5 and 5/5 at high inoculum levels and 0/5 and 0/5 for the uninoculated samples for cantaloupe peels runs 1 and 2. Similarly, *L. monocytogenes* was culturally recovered from 15/20 and 13/20 at the low and 4/5 and 5/5 at the high inoculum and 0/5 and 0/5 for uninoculated runs 1 and 2, respectively. The first cheese run had equivalent results for positive qPCR screen and cultural recovery with 20/20 at low, 5/5 at high and 0/5 for uninoculated samples. In the second run 13/20 at low, 5/5 at high and 0/5 uninoculated samples had *L. monocytogenes* detected by qPCR. Culturally, *L .monocytogenes* was recovered from 19/20, 5/5 and 0/5 at low, high and uninoculated levels, respectively.

Significance: Rapid *Listeria* species and specifically *L. monocytogenes* detection from contaminated food could streamline and increase sample throughput.

P2-61 Comparison of 3M Molecular Detection Assay *Listeria monocytogenes* and Traditional Methods to Detect *Listeria monocytogenes* from Brazilian Sushi and Sashimi

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Introduction: *Listeria monocytogenes* is a foodborne pathogen that causes listeriosis with increasing outbreak reports. Detection through traditional methods in food and environment is labor intensive and requires several days for conclusive results.

Purpose: The purpose of this study was to conduct a comparative study between the 3M Molecular Detection System (MDS) and Traditional Method, supported by Polymerase Chain Reaction (PCR) to determine the sensitivity, specificity, false positive and false negative rates.

Methods: Eighty naturally contaminated seafood samples - (*n*=40) sushi and (*n*=40) sashimi - were analyzed, according to the 3M MDA *L. monocytogenes* protocol (enrichment lysis, amplification and detection) and the Food and Drug Administration (FDA) protocol (enrichment, isolation in selective solid and differential agar media, and confirmation by biochemical tests). PCR was used to confirm the genus for colonies obtained by standard methodology.

Results: 3M MDA *L. monocytogenes* detected ten positive samples out of the eighty naturally contaminated samples (5 sushi, 5 sashimi). Presumptive positives were confirmed by the conventional method using biochemical tests. The methods showed sensitivity and specificity of 100% and 0% rates of false positives and false negatives when compared to the FDA methodology.

Significance: Sushi and Sashimi are very popular in Brazil and due to the Olympic games that will happen in 2016, thousands of foreign visitors will arrive in the country. It is crucial to detect critical risk products and apply control measures to achieve food safety in ready to eat food. In this study, it was observed that 12.5% of the samples tested were positive for this important pathogen. The use of a rapid, sensitive and specific method for the detection of *L. monocytogenes* can promote public health, supporting regulatory agencies in controlling the consumption of potentially contaminated food with lower time to results and the same reliability as standard methods.

P2-62 Development of *Listeria monocytogenes* Enumeration Method Using FSIS Guidelines in Comparison with FDA BAM *L. monocytogenes* Detection and Enumeration

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Introduction: *Listeria monocytogenes (L. mono)* is a foodborne pathogenic bacteria commonly associated with listeriosis. Listeriosis is associated with poorly prepared and/or handled foods, affecting individuals with weakened immune systems. Enumeration testing can help determine the extent of *L. mono* contamination by providing a direct measure of viable cell counts.

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Purpose: The purpose of this study was to develop a simple, cost effective enumeration method for *L. mono* using methodology from the United States Department of Agriculture, Food Safety and Inspection Service Microbiology Laboratory Guidebook (FSIS MLG). The goal was to show that *L. mono* enumeration using FSIS MLG methodology was equivalent to results obtained using the Bacteriological Analytical Manual (FDA BAM) method.

Methods: *L. mono* was enumerated in naturally and artificially contaminated ice cream, soft cheeses, and deli sandwiches. A side by side enumeration was performed using most probable number (MPN) analysis.

Results: Twenty samples were analyzed on both methods (n=20). Eight samples had the same MPN result on both methods, seven of these eight were <0.3 MPN/g. Four of the samples had a higher MPN/g on the FSIS method. Eight of the samples had a higher MPN/g on the BAM method. The difference in the number of tubes positive was only greater than one tube per sample on three samples.

Significance: Enumerating *L. mono* will provide valuable information to the food industry about the extent of *L. mono* contamination in manufacturing and distributing facilities. The FSIS MLG enumeration has the advantage of being a quicker, easier method while using fewer materials throughout confirmation.

P2-63 Monophyletic *E. coli* O157:H7 Population Spikes in Cattle Herds Observed in California's Central Valley

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) is a foodborne pathogen capable of causing life-threatening disease. In particular, serotype O157:H7 is associated with disease complications. Ruminants, such as cattle, are a natural reservoir of the pathogen and large herds of cattle are located near fields used to grow crops for raw consumption across the U.S., which presents potential public health risks.

Purpose: To gain insight into O157:H7 population diversity and dynamics, we performed a survey of 20 herds across California's Central Valley. This information is important for both molecular epidemiology and food-safety protocols, particularly regarding slaughter and harvest.

Methods: The survey of cattle was performed by obtaining isolates from individual fecal samples in 20 herds, around 40 samples per visit. Genome sequences were obtained using next-generation sequencing (Illumina MiSeq).

Results: A total of 39 herd visits were made, and 12 visits returned with samples positive for O157:H7. These 12 visits were from 9 of 20 herds visited and produced 85 O157:H7 isolates. E. coli O157:H7 isolates from herds with a high rate of isolation (>30%) had a single monophyletic lineage to which most isolates belonged. A follow-up survey from one of the farms that had a high isolation rate (87.5%), performed 11 months later, showed a highly reduced O157:H7 isolation rate (5.2%). The formerly highly prevalent lineage, however, was still present. Most of the isolates were genetically similar to clinical isolates from known O157:H7 outbreaks.

Significance: Cattle herds may go through temporary periods where O157:H7 is shed at a high prevalence throughout the population, meaning that these times, if avoided for slaughter or harvest in downstream fields, could lead to reduced risks of contamination with the pathogen. O157:H7 strains with the potential to cause severe disease are present in many herds in California's Central Valley.

P2-64 Isolation, Identification and Characterization of *Escherichia coli* O157:H7 from Cattle in Xinjiang of China

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Introduction: Cattle are a primary reservoir of *Escherichia coli* O157:H7. Preventing contamination of the food and environment with *E. coli* O157 are important measures to protect human health. Xinjiang, China, produces large quantities of beef, but the information about *Escherichia coli* O157:H7 in livestock in this part of the world are limited.

Purpose: To determine the prevalence and virulence profiles of *Escherichia coli* O157:H7 in beef cattle in Xinjiang Province.

Methods: A total of 901 beef cattle fecal samples were collected from beef cattle reared on 11 farms located in Western Xinjiang, China. Immunomagnetic beads, sorbitol-MacConkey (SMAC) agar and 4-methyl-umbelliferyl-beta-D-glucuronide (MUG) were used to isolate *E. coli* O157:H7, while PCR and biochemical tests were used for molecular characterization. Isolates were tested for s*tx1, stx2, eaeA, tccp* and *Hly* genes by PCR and subtyped by pulsed-field gel electrophoresis (PFGE).

Results: *Escherichia coli* O157.H7 was isolated from 8 of 901(0.89%) fecal samples from beef cattle in 5 of 11 herds (45.5%). Three isolates were negative for *stx*1 and *stx*2 and all other virulence genes tested. One isolate had *stx*1 and *stx*2 and the remaining four isolates were *stx*2 only. Two of the *stx*2 only isolates did not have other virulence genes. Thus, only three isolates, (one $stx1^{+}$,$stx2^{+}$ and two $stx2^{+}$ isolates) had the complement of other virulence genes Hly^{+}, $tccp^{+}$ and $eaeA^{+}$. The eight isolates represented six different unique PFGE patterns. Three strains isolated from different herds in different regions had identical PFGE patterns.

Significance: This is the first report of *E. coli* O157 isolation from live cattle in far Western China. Although this finding is not surprising, it does provide important baseline data about the global epidemiology of this important foodborne pathogen and the basis for future research.

P2-65 Prevalence and Epidemiological Analysis for *Listeria monocytogenes* Isolates from Farms in S. Korea

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❖ ***Developing Scientist Competitor***

Introduction: Since *Listeria monocytogenes* is a zoonotic pathogen, causing listeriosis, the pathogen can be transmitted from animal in farm to human. Thus, prevalence of *L. monocytogenes* in should be investigated.

Purpose: The objective of present study was to investigate the prevalence and genetic correlations of *L. monocytogenes* in cattle and pig farms in S. Korea.

Methods: Feces, soil, silage (cattle farm), and sludge (pig farm) samples (n=2,018) were collected from 25 cattle and pig farms, which were visited 2-3 times. *L. monocytogenes* were isolated by plating the samples on PALCAM agar, and presumptive colonies were identified by amplifying *Listeria*-specific genes (*hly* and *prs*) by PCR and by 16s rRNA analysis. All *L. monocytogenes* isolates were then serotyped by multiplex PCR and agglutination assay. Virulence genes (*inlA*, *inlB*, *actA*, *plcB*, and *hlyA*) were identified by PCR, and genetic correlations were also evaluated by pulsed-field gel electrophoresis (PFGE) patterns digested with *Asc*I restriction enzymes.

Results: Of 2,018 farm samples, just three samples (0.15%) were *L. monocytogenes* positive and isolated from soil samples in same farm. Even though the samples were from same farm, serotypes of the isolates from farms were 3a (one isolate) and 4ab (two isolates), which are low pathogenic. In PFGE patterns of *L. monocytogenes*, genetic correlations between same isolates (4ab) 100%, but the genetic correlation between different serotypes was only 26.3%.

Significance: These results indicate that there is very low prevalence of *L. monocytogenes* in cattle and pig farms. Thus, the possibility of transmission of the pathogen from farm to people is very low.

P2-66 Contamination of Post-harvest Poultry Products with Multidrug-resistant *Staphylococcus aureus* in the Maryland-Washington D.C. Metro Area

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Introduction: *Staphylococcus aureus* has historically been a serious human pathogen, and during recent decades it has become a more serious threat due to acquisition of antibiotic resistance.

Purpose: In the present study, we aimed to investigate the contamination level and antibiotic resistance pattern of *S. aureus* among industrial, antibiotic-free organic/pasture grown and farmers markets poultry products from the Maryland-Washington DC metro area.

Methods: A total of 96 poultry whole carcass samples including 32 samples from 7 farmers markets, 28 samples from 3 organic retail supermarkets and 36 samples from 3 conventional retail supermarkets from the Maryland-Washington DC metro area were collected over a period of eight months starting from February to September of 2014.

Results: A total of 24 *S. aureus* isolates were recovered from 96 whole poultry carcass samples and the prevalence of *S. aureus* were 25.0%, 14.29%, and 33.3% in retail poultry meats collected from farmers markets, organic and conventional retail supermarkets, respectively. Both single and multi-drug-resistant isolates were detected in 58.3% (7/12) isolates from conventional retail meat products but none from farmers markets or organic retail meat isolates. Conventional retail meat isolates were found to be resistant to both erythromycin (50.0%) and tetracycline (58.3%). We also detected an MRSA isolate harboring *mecA* gene in conventional retail meat which showed co-resistance towards erythromycin, tetracycline, and vancomycin.

Significance: Although this was a small study in a limited geographical area, this study serves as an indication that *Staphylococci* including MRSA are present in retail chicken meat. Additional studies are needed in order to assess the risk of MRSA colonization in poultry gut and the workers who handle raw meat.

P2-67 The Association between Non-foodborne Exposures and the Occurrence of Non-typhoidal Salmonellosis in Tennessee

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❖ ***Developing Scientist Competitor***

Introduction: Salmonellosis is a growing public health problem worldwide. The estimates of non-typhoidal salmonellosis (NTS) is approximately 1.4 million/year occurs in the U.S, costing several billion dollars annually. Although the ingestion of contaminated food is considered as one of the major sources of NTS, non-foodborne and/or environmental exposures may also play major roles to transmit NTS.

Purpose: The purpose of this study was to explore the associations between the non-foodborne (including environmental) exposures and the occurrence of NTS caused by *Salmonella* serotypes Newport, Javiana, and Mississippi in a FoodNet catchment area (Tennessee).

Methods: Data from FoodNet and Tennessee Department of Health comprising of 3,470 laboratory confirmed cases of NTS from 2011-2014 were used to calculate the incidence rates in Tennessee. Case-case analyses were conducted for 1,624 laboratory confirmed cases of NTS. NTS caused by serotypes Newport, Javiana, and Mississippi were considered as cases whereas the comparison group consist of with other *Salmonella* serotypes.

Results: After adjusting for the demographics such as age, sex, race, ethnicity, and the month and year of disease onset, NTS was found to be significantly associated with the two exposure variables (1) contact with chicken and (2) contact with pet that had diarrhea. The odds of NTS was 1.9 (95% CI: 1.10- 3.32, P=0.02) times higher for those who came in contact with chicken and 2.1 times (95% CI: 1.14- 3.90, P=0.01) higher for those who came in contact with pet that had diarrhea.

Significance: This study reveals the incidence of NTS caused by *Salmonella* serotypes Newport, Javiana, and Mississippi serotypes increased significantly in Tennessee. Furthermore, contact with certain types of animals is a major preventable route of transmission of NTS. In addition, continued efforts to increase awareness and practice of hand hygiene are needed to prevent the occurrence of the infection.

P2-68 2015 Multistate Outbreak of *Salmonella* Paratyphi B Variant L(+) tartrate(+) and *Salmonella* Weltevreden Infections Associated with Imported Frozen Raw Tuna

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Introduction: In 2015, FDA, CDC, and state and local officials investigated a multistate outbreak of *Salmonella* Paratyphi B variant L(+) tartrate(+) and *Salmonella* Weltevreden infections linked to consumption of frozen raw tuna used as a sushi ingredient.

Purpose: To describe outbreak response activities and discuss conclusions drawn from the investigation.

Methods: A case was defined as infection with the outbreak strains of *Salmonella* Paratyphi B variant L(+) tartrate(+) or *Salmonella* Weltevreden occurring between 3/1/2015 – 7/31/2015. Investigators analyzed case-patient interview data to identify common foods. Records documenting the import and distribution of tuna to restaurants associated with illnesses were collected to determine the source of the tuna used to make sushi reported by case-patients. Investigators collected and analyzed product samples.

Results: Sixty-nine cases were reported from 11 states. Raw tuna in sushi was consumed by 98% (46/47) of case-patients interviewed. Investigators traced frozen raw tuna (ground or chunk) used at 8 restaurants linked to 16 illnesses. Distribution records identified Importer A as a common source of frozen raw tuna that had been distributed to 7 of the 8 restaurants. Minnesota investigators recovered outbreak strains of *Salmonella* Paratyphi B variant L(+) tartrate(+) and *Salmonella* Weltevreden from intact samples of frozen raw tuna distributed by Importer A. Traceback analysis determined that the contaminated tuna was sourced from Indonesia. Importer A voluntarily recalled product it had received from Indonesia and distributed from 5/9/2014 – 7/9/2015.

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Significance: Frozen raw tuna imported from Indonesia was implicated as the source of this outbreak. Since 2007, four *Salmonella* outbreaks have been linked to raw imported tuna, highlighting the risks associated with raw seafood consumption and raising the possibility that seafood harvest/processing practices used by foreign firms may have contributed to contamination of tuna products intended for raw consumption.

P2-69 Estimating the Burden of Foodborne Illness for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in Japan, 2006–2013

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Introduction: In Japan, the numbers of food poisoning incidence and cases are reported mandatory; however, these do not exactly reflect the real burden of foodborne illnesses due to the passive surveillance nature. We have been estimating the real burden of foodborne diseases for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in Japan.

Purpose: To estimate the burden of foodborne illnesses associated with three pathogens in Japan, by making use of the laboratory confirmed numbers of infections.

Methods: Data on laboratory-confirmed infections of three pathogens were collected from clinical laboratories that test stool samples submitted from all over Japan or from Miyagi Prefecture, from January 2006 to December 2013. The physician consultation rate and the stool submission rate were estimated from telephone population surveys conducted for whole of Japan and for Miyagi prefecture. We merged the telephone survey data conducted in 2014 with previous data. Each estimate was introduced into the Monte-Carlo simulation model as a probability distribution, which was run for 10,000 iterations.

Results: The estimated mean numbers per year of foodborne illnesses for *Campylobacter*, *Salmonella* and *V. parahaemolyticus* in whole of Japan were 4.8 to 11.3 million, 1.0 to 2.3 million, and 86 to 367 thousand during 2006 to 2013, respectively. Those estimated for whole of Japan from data on Miyagi prefecture were 0.64 to 1.6 million, 78 to 190 thousand, and 7 to 63 thousand during 2006 to 2013, respectively. The numbers of reported foodborne illnesses per year in Japan during 2006 to 2013, for *Campylobacter*, *Salmonella* and *V. parahaemolyticus*, were 1,600 to 3,100, 670 to 3,600 and 90 to 1,300, respectively.

Significance: These data reveal a significant difference in numbers and trends between our estimates of burden of foodborne illnesses and the reported foodborne disease cases associated with three pathogens. Need for continuing active surveillance system to complement the present passive surveillance is strongly suggested, in order to identify and prioritize food safety issues more precisely and to monitor the effectiveness of risk management options.

P2-70 A Summary of Foodborne Illness Outbreaks Investigated by FDA's Coordinated Outbreak Response and Evaluation Network, August 2011 to December 2015

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Introduction: The Coordinated Outbreak Response and Evaluation Network (CORE) of the Food and Drug Administration, formed in August 2011, is a multidisciplinary team that evaluates, investigates, and guides prevention efforts of foodborne illness outbreaks. Working with its federal, state, and local colleagues, CORE has investigated 108 outbreaks attributed to FDA-regulated products.

Purpose: The purpose of the analyses is to spotlight CORE's accomplishments as well as display and summarize FDA outbreak data.

Methods: The 108 outbreaks, occurring August 2011 to December 2015, were analyzed by product category; year; pathogen; and number of illnesses, hospitalizations, and deaths using data from the FDA CORE Outbreak Database and the Emergency Operations Network database.

Results: The analyses found that since August 2011, there have been 7,181 illnesses, 1,403 hospitalizations and 69 deaths due to these foodborne illness outbreaks. The majority of outbreaks were attributed to produce (*n*=42), followed by seafood (*n*=21) and processed food (*n*=12). Illnesses were also predominantly caused by outbreaks associated with produce (*n*=4,451), followed by seafood (*n*=608) and processed foods (*n*=511). Outbreaks associated with produce were attributed to 47 deaths, followed by dairy (*n*=15). These deaths are primarily due to foods contaminated with *Listeria monocytogenes* (77%), whereas the majority of illnesses are attributed to *Salmonella* infections (53%). In response to these outbreaks, CORE has worked in partnership with the CDC, USDA, and all five FDA regional and 20 district offices. CORE also allied with at least 67 State public health/agriculture agencies, as well as international partners.

Significance: A coordinated effort to respond to foodborne illness outbreaks enables FDA CORE to streamline and more quickly identify, respond to, and prevent outbreaks. CORE continues to collaborate with its federal, state, and international partners to refine its processes to detect, investigate and prevent outbreaks as efficiently as possible to secure a safer food supply.

P2-71 It Won't Happen to Me: Unrealistic Food Safety Optimism among People Living with HIV in Beijing

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Developing Scientist Competitor

Introduction: People living with HIV (PLWH) have an increased risk of foodborne diseases (FBDs) due to damage by HIV on their immune system. There are few data concerning their awareness of this vulnerability and no data from China.

Purpose: To examine self-perceptions of vulnerability to FBDs in the domestic environment of PLWH in Beijing.

Methods: A cross-sectional study using face-to-face interviewers was conducted in 2015 with 200 PLWH recruited through convenience sampling from a Beijing hospital. A structured questionnaire asked participants about their food/drink storage, cooking, and consumption practices at home. Self-perceptions of both their own and other PLWH's risk for domestic FBDs were measured using a Likert scale from 1 (Not at all likely) to 5 (Extremely likely). A self-perceived relative risk score for each participant was obtained by subtracting scores for perceptions of "others'-risk" from "own-risk." Risky domestic food behavior was measured using a 5-item Likert scale, which included 22 behavior questions.

Results: When asked about their likelihood of contracting a FBD in their home: 88 (44%) responded not at all likely, 104 (52%) slightly likely, and 8 (4%) moderately likely. Scores measuring the participants' domestic food behaviors revealed that both those responding "no-risk" (2.59±0.43) and those responding "moderate/slight-risk" (2.50±0.42) engaged in unsafe food practices, but with no statistically significant differences in food behavior between the two (t=1.482, *P*=0.14).Relative risk scores indicated that nearly all participants (98%) perceived themselves to be equally (53.5%) or less (44.5%) vulnerable to domestic FBDs than other PLWH.

Significance: These data revealed that many PLWH in Beijing, China, perceived themselves as no or low risk for FBDs and at lower risk than other PLWH. To be successful, interventions to improve domestic food safety in China must first help PLWH to perceive and understand their personal risk for acquiring a FBD.

P2-72 Antimicrobial Susceptibility Patterns of *Enterococcus* in Cattle and Geese Feces and Their Shared Soil Environment

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Introduction: Antimicrobial resistance is a global concern. The relatedness and persistence of fecal microorganisms in migratory birds, soil and production animals in which they transiently cohabitate over time has been reported infrequently. *Branta canadensis* (Canada goose) populations are becoming resident across many communities and animal production facilities. This presents an interesting dynamic for the study of bacteria that are commonly carried between species, including humans. Cattle operations are ideal locations for geese to co-exist.

Purpose: The purpose of this study was to longitudinally follow the antimicrobial susceptibility patterns of *Enterococcus* species throughout an environment shared by dairy cattle and a transient population of *Branta canadensis* (Canada goose).

Methods: Approximately 5 g of feces were collected over a one year period from dairy cattle (*n*=275) and Canadian geese *(n*=242*).* Additionally, *n*=220 10g soil samples were collected from defined pasture plots representing areas where both cattle and geese mingled. Selective culture methods were used to isolate Enterococcus, and antimicrobial susceptibility was determined using broth microdilution (Vitek, BioMerieux, Marcy l'Etoile). Interpretations were based on CLSI guidelines.

Results: *Enterococcus* species from cattle (*n*=152), geese (*n*=195), and soil (*n*=152) were tested for susceptibility. The predominant species recovered was *E. hirae* (35%), followed by *E. faecium* (25%) and *E. casseliflavus* (18%). Approximately 94% of *Enterococcus* isolates were resistant to at least one antimicrobial. Multidrug resistant *Enterococcus* (≥ 3 antimicrobials) were obtained from 49%, 49%, and 47% of soil, geese, and cattle samples, respectively. The predominant MDR pattern of *E. faecium (*51%; n=88 isolates) was to clindamycin, enrofloxacin, marbofloxacin, and trimethoprim/sulfamethoxazole, and was observed in all sample types.

Significance: The presence of multidrug resistant *Enterococcus* sharing similar susceptibility patterns could be demonstrated in migratory geese, associated cattle and their shared soil environment. This suggests that migratory geese play a role in the dissemination of antimicrobial resistant bacteria.

P2-73 Microbial Diversity and Associations between Cattle and Geese Feces and Their Shared Soil Environment

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Introduction: Understanding the mechanisms of pathogen transmission and the dissemination of antimicrobial resistance genes throughout ecological systems is important to combat emerging antimicrobial resistance. Migratory birds are known reservoirs for antimicrobial resistant (AR) organisms and frequent cattle pasture operations. The relatedness between microbiota from migratory birds' feces, soil and the feces from production animals co-mingled in their transient environment over time is lacking.

Purpose: The purpose of this study was to longitudinally follow the prevalence and relatedness of microorganisms throughout an environment shared by dairy cattle and a transient population of *Branta canadensis* (Canada goose).

Methods: Feces (~5 g) from dairy cattle (closed herd, *n* = 25, collected by rectal palpation) and Canada geese (*n* = 22, collected from ground), as well as soil samples (~10 g, *n* = 20) from a defined pasture plot were collected approximately once per month for one calendar year (*n* = 11 collections). Standard diagnostic laboratory culture methods were used to isolate *Enterococcus, Escherichia coli,* and other gram-negative organisms. Isolates were confirmed using MALDI-TOF mass spectrometry.

Results: *Enterococcus* species were readily obtained from cattle (*n* = 223), geese (*n* = 195), and soil (*n* = 152) samples. The predominant species was *E. hirae* (35%), with *E. faecium* (25%), *E. casseliflavus* (18%) and *E. faecalis* (3%) also recovered. The distribution of species was similar for all sample types. *Escherichia coli* also was frequently recovered from cattle (*n* = 230), geese (*n* = 223), and soil (*n* = 220). The distribution of additional gram-negative organisms including *Citrobacter, Enterobacter,* and *Acinetobacter* species varied, but was more diverse in soil than either cattle or geese feces.

Significance: The presence of microorganisms of public health importance across all sources suggests the likelihood that the exchange of microbial populations is occurring between migratory geese, associated cattle and their shared soil environment.

P2-74 A Comparison of Antimicrobial-susceptibility Patterns of *Escherichia coli* Isolated from Cattle, Geese and Soil

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Introduction: Recent global reports indicate that *Escherichia coli* are rapidly expressing resistant to drugs of last resort. This is particularly worrisome as no new antimicrobials are forthcoming. In order to develop appropriate mitigation strategies, it is important that we understand pathogen transmission and the dissemination of resistance genes throughout ecological systems. *Branta canadensis* (Canada goose) have previously been recognized as a reservoir for antimicrobial resistant organisms. Cattle operations are an excellent model system in which to study the relatedness and persistence of microorganisms in cattle, Canadian geese, and their shared soil environment.

Purpose: The purpose of this study was to longitudinally follow the antimicrobial susceptibility patterns of *E. coli* isolates throughout an environment shared by dairy cattle and a transient population of *Branta canadensis* (Canada goose).

Methods: Over a 12 month period, samples (~5 g feces) were collected from dairy cattle (*n*=275) and Canada geese (*n*=242). Soil samples (*n*=220; 10g) from defined pasture plot areas were also collected. Selective culture was used to isolate *Escherichia coli*, and antimicrobial susceptibility was determined using broth microdilution (Vitek, BioMerieux, Marcy l'Etoile). Interpretations were based on CLSI guidelines.

Results: Antimicrobial susceptibility was determined for *Escherichia coli* isolated from cattle (n=230), geese (n=201), and soil (n=123). The majority (81.4%) of isolates exhibited no resistances to the panel of antimicrobials evaluated. Seven (1.2%) isolates exhibited extended spectrum beta-lactamase (ESBL) activity. The most common resistance pattern (4.7% of isolates) was to ampicillin, piperacillin, and tetracycline; this pattern was found in *E. coli* from cattle, geese and soil.

Significance: We demonstrated the presence of multidrug resistant *E. coli,* including ESBLs over time in a shared environment of cattle and migratory geese. The possibility of further transmission from migratory birds warrants further study.

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P2-75 Synanthropic Wildlife Associated with Livestock Production as Carriers of High Priority Antimicrobial Resistances

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Introduction: At the wildlife-livestock interface, wildlife have been found to carry antimicrobial resistant (AMR) bacteria and genes, which may promote the development and exchange of AMR between livestock and the environment. Thus, wildlife incursion into agricultural facilities that are major foci of AMR, such as concentrated animal feeding operations (CAFOs), is of particular concern. Efforts to understand the prevalence and extent of AMR in specific wildlife carriers are needed to profile AMR emergence, evaluate transmission dynamics, and identify mitigation points for wildlife managers and producers.

Purpose: This study assessed AMR indicator bacteria (*Escherichia coli* and *Enterococcus* sp.) collected from CAFO-associated wildlife (primarily rodents and raccoons) with similar bacteria from cattle and the CAFO environment, emphasizing the detection of multiple types of AMR classified by the United States Centers for Disease Control and Prevention as "Urgent, Serious, and Concerning Threats."

Methods: A total of 726 wildlife fecal, 195 cattle fecal, and 154 environmental samples were collected from five CAFOs located in Northern Colorado. An isolation procedure which incorporated sub-minimal inhibitory concentrations of high-priority antibiotics into microbiological growth media was used to screen these samples for the AMR indicator bacteria. Species identification of isolates was confirmed by MALDI biotyping. Antimicrobial susceptibility determinations were achieved using the disk diffusion assay to 18 antibiotics for *E. coli* and 13 antibiotics for *Enterococcus* sp. isolates.

Results: From the fecal and environmental samples, MALDI Biotyping confidently identified (Biotyping score ≥ 2.0) 555 AMR indicator bacteria. The extent of AMR was associated with host organism, CAFO, and the specific *Enterococcus* sp. Comparison of antimicrobial susceptibly biosignatures indicated that specific isolates were circulating between cattle, wildlife, and among CAFO sites.

Significance: This study demonstrates that synanthropic wildlife may contribute to the exchange and dissemination of AMR in livestock production.

P2-76 Responding to an Outbreak of *Salmonella* Poona Infections Associated with Cucumbers from Mexico: A Collaboration between the FDA, CDC, DoD, and State Partners

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Introduction: On August 18, 2015, the Food and Drug Administration (FDA) was notified, through PulseNet, of 32 people infected with *Salmonella* Poona of an indistinguishable pulsed-field gel electrophoresis (PFGE) pattern in 13 states. Cucumbers were identified as the suspected food vehicle. As of January 4, 2015, 884 people infected with the outbreak strain of *Salmonella* Poona have been reported from 39 states, with 189 hospitalizations and four deaths.

Purpose: State, local, and federal government agencies responded quickly to determine the source of *Salmonella* Poona associated with cucumbers.

Methods: Through collaboration with the Center of Disease Control and Prevention (CDC), Department of Defense, and state investigational partners, FDA identified six sub-clusters of cases. Federal and state partners collected documents and cucumber samples throughout the supply chain. Whole genome sequencing analysis was conducted on *Salmonella* Poona isolates obtained from clinical cases and suspected cucumbers.

Results: Traceback lead to a common distributer for all six sub-clusters of cases. On September 4, 2015, Distributer A of San Diego, California issued a voluntary recall of all cucumbers supplied to the United States from August 1, 2015 through September 3, 2015, after being presented with information by California Department of Public Health, FDA and CDC. Twenty-three of the cucumber samples sourced from Grower A of Baja California, Mexico, yielded *Salmonella* Poona isolates matching the outbreak strain. Whole genome sequencing analysis suggested a common source of contamination. FDA placed Grower A on two separate Import Alerts to prevent future shipments of the firm's cucumbers from entering the United States.

Significance: The collaborative efforts of federal, state, and local partners, led to the identification of the source of the outbreak, isolation of the outbreak strain from cucumber samples, a voluntary recall by the distributer, and prevention of further shipments of suspect cucumbers from entering the United States.

P2-77 Large-scale Bioinformatic and Phylogenetic Analysis of *Listeria monocytogenes* Genomes Reveal Select InlA Genotypes Associated with Virulence and Transmission in Ecological Food Niches

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Introduction: The foodborne pathogen, *Listeria monocytogenes,* exhibits fitness in diverse ecological niches, including mammalian hosts, agricultural, and food environments. Human listeriosis is predominantly associated with contaminated ready-to-eat (RTE) deli meats and cheese, however recent outbreaks associated with produce (e.g., sprouts) and fruit (e.g., caramel apples) indicate agricultural environments may serve as an emerging ecological niche for multiplication and transmission of pathogenic *L. monocytogenes*. Internalin A, is encoded by *inlA* and mediates host colonization. Several mutations leading to truncated InlA protein are associated with attenuated virulence phenotypes and are enriched among food isolates but rare among clinical isolates.

Purpose: Our study sought to analyze distinct InlA functional and genotypic variants associated with *L. monocytogenes* from food and clinical sources.

Methods: We utilized phylogenetic and bioinformatic methods to analyze distribution of distinct InlA functional and sequence variants identified among 2209 genomes representing isolates from various sources.

Results: InlA non-functional subtypes were significantly (P<0.001) associated with food categories (dairy, produce, and ready-to-eat (RTE)). Among lineage I and lineage II, respectively, functional InlA variants were significantly enriched among RTE isolates (17.7% and 43.8%; P<0.0001), while functional subtypes were enriched among produce (99.1% and 91.3%; P<0.0001) isolates. We further analyzed the distribution of InlA PST subtypes within *L. monocytogenes*. Overall, 252 unique InlA PST were identified among strains from lineage I (n=45) and lineage II (n = 113). Individual InlA PST variants were distinct for each lineage and isolate source for lineage I strains. Four InlA PST were enriched among lineage I strains from clinical (77.8%), food (44.5%) and environmental (34.2%) sources; these InlA variants also represented the most frequent subtype present among all produce and dairy isolates.

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Significance: Our results indicate *inlA* genetic variation may contribute to distinct ecological preference and multiplication and transmission of pathogenic *L. monocytogenes* via host-dependent dispersal mechanisms in agricultural environments.

P2-78 Microbiological Quality and Safety of Fresh Produce and an Assessment of Post-harvest Practice of Vendors at West Virginia and Kentucky Farmers' Markets

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❖ ***Developing Scientist Competitor***

Introduction: Fresh produce sold at farmers' markets (FMs, <$500,000 annually) raises food safety concerns due to their exemption of Food Safety Modernization Act. No information is available regarding microbial quality/safety of produce at West Virginia- and Kentucky-FMs.

Purpose: This study aims to evaluate the microbiological quality/safety of 5 different fresh produce from WV- and KY-FMs, and assess the post-harvest practices of vendors at Morgantown-WV-FM.

Methods: A total of 212 produce samples including tomatoes (64 samples, 13 vendors), green peppers (54 samples, 11 vendors), cucumbers (35 samples, 5 vendors), cantaloupes (16 samples, 2 vendors) and spinaches (43 samples, 8 vendors) were randomly purchased from two Morgantown-WV-FMs and one Bowling Green-KY-FM during fall 2015. Buffered-peptone-water (BPW) processed samples were analyzed for the aerobic-plate-counts (APCs), total-coliform-counts (TCCs), and yeast/molds population on petrifilms, and were secondly-enriched in Rappaport-Vassiliadis (*Salmonella enterica* spp.) and UVM broth (*Listeria* spp.) followed by streak-plating onto XLD (*Salmonella enterica* spp.) and Modified-Oxford agar (*Listeria* spp). Presumptive *Salmonella* and *Listeria* colonies were confirmed by API20E-kit+qPCR (*Salmonella*-InvA gene) and *Listeria*12L-kit+multiplex-PCR, respectively. A follow-up survey (20 questions) was conducted to assess the post-harvest practices of 28 vendors at Morgantown-WV-FM. Data (3 repeats/10-20 samples/repeat) were analyzed using the ANOVA and Chi-Square test of SAS.

Results: Among 212 samples, APCs, TCCs, and yeast/molds were 3.72-5.63, 3.67-5.47, and 3.07-4.13 log CFU/g, respectively, and spinach significantly contained the highest (P<0.05) population of APCs, TCCs, and yeast/molds. *Salmonella enterica* spp. was detected on 10.9% (7/64) of tomatoes, 18.5% (10/54) of peppers, 56.3% (9/16) of cantaloupes, and 18.6% (8/43) of spinach. Only 3.78% (8/212) of samples were confirmed for *Listeria* spp., and 50% (4/8) were identified as *L. monocytogenes*. Survey showed that 74% (20/27) venders washing produce with only 9% (2/23) using sanitizers/antimicrobials, and 50% (7/14) did not refresh sanitizers.

Significance: Results showed *Salmonella* and *Listeria* present on fresh produce at local FMs, and indicated that developing post-harvest protocols to control foodborne pathogens and developing Good Agriculture Practices training material/courses are important for FM vendors.

P2-79 Microbial Quality of Leafy Greens and Herbs Purchased from Farmers' Markets in Virginia and North Carolina

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Introduction: The number of registered farmer's markets has nearly tripled in the last 15 years. Fresh produce accounts for over 80% of the food sold at these markets. Previous work shows that leafy greens and herbs tend to have a higher microbial load amongst fresh produce commodities.

Purpose: The purpose of this study was to assess microbial quality of different leafy green and herb varieties sold at farmers' markets in Virginia and North Carolina.

Methods: Fourteen different varieties of leafy green and herb samples (n=256) were collected from NC and VA farmers' markets between June and October. Each sample was tested for total aerobic plate count (APC), coliforms, generic *E. coli* and Enterococci using 3M Petrifilm and KF Streptococcal agar. To detect pathogens, samples were enriched in selective media and T-streaked onto selective agar to identify presumptive positives colonies.

Results: Arugula and salad mix were highest in all counts, including coliform (3.97 and 4.39 CFU/g), *E. coli* (0.27 and 0.20 CFU/g) and Enterococci counts (3.79 and 3.41 CFU/g). Cilantro had the highest APC count of all varietals (7.70 log CFU/g), while romaine lettuce and basil had the lowest (6.25 and 6.22 log CFU/g, respectively). Varieties with no detectable *E. coli* were butter head lettuce, red leaf lettuce, purslane, cilantro and basil. Enterococci counts were highest in red leaf lettuce and cilantro (4.13 and 3.74 log CFU/g, respectively) and lowest in butter head lettuce and basil (2.88 and 2.60 log CFU/g, respectively). There were generally no differences in microbial counts associated with vendor where product was purchased.

Significance: While leafy greens and herbs inherently contain high microbial loads, the results suggest that certain varietals may be at higher risk for contamination. Specific handling practices may contribute to this risk.

P2-80 Statistical Analysis of the Microbial Quality of Fresh Produce from University Foodservice Facilities

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❖ ***Developing Scientist Competitor***

Introduction: Fresh produce is an important part of a healthy diet. Microbial indicator organisms can be used to assess fresh produce microbiological quality, (and potentially the safety) because foodborne pathogens are rarely present.

Purpose: This study characterized the microbial quality of ready-to-eat produce items in university dining facilities using data collected over twelve years, to determine if a unified microbial quality standard for all fresh produce would be possible. The relationship between indicator organisms and foodborne pathogens was also explored.

Methods: Data were obtained from 955 produce samples taken from university dining facilities. Total aerobic count, presumptive and confirmed total coliform, and fecal coliform counts were determined using FDA BAM methods. Each sample was also tested for the presence of generic *E. coli*, pathogenic *E. coli, Salmonella, Staphylococcus aureus, Listeria monocytogenes* and *Bacillus cereus*. Statistical analysis was performed for any produce items where n>30.

Results: Data from thirteen different produce types met the criteria for inclusion (n>30). Apples had the lowest median total aerobic count (2.8 log CFU/g), while spinach had the highest (6.7 log CFU/g). Apple, cauliflower and mixed lettuce had the lowest median of total coliform counts, while bell peppers had the highest (3.0 log CFU/g). Bell pepper had the lowest median *B. cereus* counts (1.7 log CFU/g), while spinach had the highest with a median of 2.8 log CFU/g. *Staphylococcus aureus* was isolated in 2.5% of samples, *Escherichia coli* from 1.4% of all the samples, and *E. coli* O157:H7

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was isolated only once. *Salmonella* spp. was isolated from 0.7% of samples, *L. monocytogenes* from 0.3% of all the samples, and *B. cereus* from 6.8% of samples.

Significance: Since levels of indicators were highly variable, development of a uniform microbial quality standard is problematic. Because foodborne pathogens were rarely isolated, it was not possible to determine suitable indicators.

P2-81 Survival of *Salmonella*, *Listeria monocytogenes*, and O157 and Non-O157 Shiga Toxin-producing *Escherichia coli* on Fresh-cut Produce during Storage at 10°C

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Introduction: Foodborne illnesses associated with contaminated fresh produce are a growing public health concern. Individuals in food insecure households are more at-risk for serious consequences of foodborne illness compared to the general population.

Purpose: This study aimed to investigate the survival of *Salmonella enterica* (SE), *Listeria monocytogenes* (LM), and O157:H7 and non-O157 Shiga toxin-producing *Escherichia coli* (STEC) on 10 types of fresh-cut produce often available in food pantries.

Methods: Fresh-cut produce items: apple, cantaloupe, carrot, cucumber, celery, onion, pepper, radish, strawberry, and tomato, were gleaned or purchased from local retailers. For each produce/pathogen trial, seven 25-g aliquots of produce were inoculated with a single-pathogen cocktail of SE (5 strains), LM (5 strains), or O157 and non-O157 STEC (11 strains) (0.025 ml inoculum) to 3-log CFU pathogen/g. Samples were stored at 10°C for up to 5 days with periodic sampling and spread-plating on appropriate agar for pathogens or native flora. Triplicate trials were conducted for each pathogen/produce combination. Log surviving cells (CFU/g) was plotted versus incubation time.

Results: There were five survival patterns: 1) Cantaloupe, celery, cucumber: no change in pathogen, native microflora increased 2 to 3 log CFU/g; 2) Carrot, pepper, radish: decrease in pathogen by 1 log CFU/g; native microflora increased by 1 to 2 log CFU/g; 3) Tomato: decrease in pathogen survival; native microflora increased by 1-log CFU/g; 4) Apple, strawberry: no change in viability of SE or STEC; no survival of LM; 5) Onions: decrease of 1-log CFU/g for pathogens, decrease in native flora counts. Levels of native microflora varied from 3 to 7 log CFU/g at time 0.

Significance: Fresh-cut produce items did not support or enhance the growth of pathogens. These results are counter to research which has shown the rapid growth of pathogens on produce items such as cantaloupe, and suggest that native microflora may play an important role in the safety of fresh produce.

P2-82 Prevalence and Characterization of *Bacillus cereus* from Ready-to-Eat Vegetables in South Korea

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Introduction: Ready-to-eat (RTE) vegetables are becoming an increasingly popular food choice. Since RTE vegetables are not commonly sterilized by heat treatment, contamination with foodborne pathogens such as *Bacillus cereus* (*B. cereus*) is a major concern.

Purpose: The objective of this study was to assess the prevalence, toxin profiles, and phenotype of *B. cereus* from RTE vegetables.

Methods: A total of 155 individually packed RTE vegetables were purchased from retail stores in South Korea. The confirmation of possession of diarrheal and emetic toxin genes such as *nheABC, hblCDA, cytK, entFM, EM1*, and *ces* was performed by using PCR assay. Biochemical tests including starch fermentation, salicin hydrolysis, hemolysis, motility tests, and lecithinase were conducted for all isolates. To assess B. cereus psychrotrophic growth characteristics, all strains were inoculated onto sheep blood agar, and plates were incubated at 7°C over a period of 21 days.

Results: We found that 70 of the 155 (45%) tested retail vegetable salad and sprout samples were positive for *B. cereus*. The *B. cereus* isolates harbored at least one enterotoxin gene. The detection rates of *nheABC, hblCDA, cytK,* and *entFM* enterotoxin genes among all isolates were 97.1%, 100%, 81.4%, and 98.6%, respectively. No strain carried the emetic toxin genes such as *EM1* and *ces*. The percentage of strains that were positive for salicin hydrolysis, starch fermentation, hemolysis, motility test, and lecithinase hydrolysis were 91.4%, 95.7%, 80%, 100%, and 100%, respectively. Only six strains (8.6%) from the 70 isolates were psychrotrophic and were able to grow at 7°C. All of the psychrotrophic isolates possessed at least 1 enterotoxin gene.

Significance: Considering the high contamination levels and the toxin profiles of *B. cereus* in RTE vegetables, the products should be evaluated by using strict microbial safety standards and post-processing control measures that ensure product safety.

P2-83 Evaluation of the Use of Shotgun Metagenomic Sequencing for Detection and Strain Level Discrimination of Shiga Toxin-producing *Escherichia coli* Contamination on Fresh Bagged Spinach

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Introduction: Consumption of fresh bagged spinach contaminated with Shiga toxin-producing *Escherichia coli* (STEC) has led to severe illness and death. Since not all STEC strains are considered human pathogens, virulence characterization of STEC strains is important. Shotgun metagenomics may provide a rapid method to detect, obtain virulence gene information, and determine strain identification and phylogenetic relatedness.

Purpose: The objective of this study was to evaluate the comprehensiveness of a metagenomics approach for detection and strain level identification of STEC on bagged spinach using pathogenic STEC strains of a variety of serotypes and Shiga toxin subtypes.

Methods: Bagged spinach was spiked with one of 12 STEC strains at a level of 0.1 CFU/g spinach and processed according to the U.S. FDA BAM protocol. Sequencing data generated from each sample was used to determine molecular serotype and STEC-specific virulence genes by BLAST analysis, identify the microbial communities present in the enriched sample using a discriminative k-mer method, and perform *E. coli* core gene SNP analysis on *de novo* assemblies of the metagenomic sequencing data.

Results: Bacterial community analysis determined that *E. coli* was a major component of the population in most samples, but molecular serotyping using the metagenomic data revealed the presence of indigenous *E. coli* in some samples. Despite the presence of additional *E. coli* strains, the serotype and virulence genes of the spiked STEC, including correct Shiga toxin subtype, were detected in 92% of the samples. *E. coli* core gene SNP analysis of the metagenomic sequencing data correctly placed the spiked STEC in a phylogeny of related strains in cases where the indigenous *E. coli* did not predominate.

Significance: Utilizing a shotgun metagenomics approach to characterize STEC contaminating bagged spinach may expedite the time necessary to ascertain the risk level to public health and response time during outbreaks.

P2-84 Quantifying Redistribution of *Salmonella* Typhimurium LT2 during Simulated Commercial Production of Fresh-cut Baby Spinach and Cilantro

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❖ ***Developing Scientist Competitor***

Introduction: Several recent outbreaks traced to baby spinach and cilantro have been hypothesized to involve cross-contamination during washing and processing.

Purpose: Consequently, this study aimed to assess the redistribution of *Salmonella* Typhimurium LT2 during pilot-scale production of fresh-cut baby spinach and cilantro.

Methods: Four inoculated:uninoculated product weight ratios (0.5:100, 1:100, 5:100, and 10:100) and three different inoculation levels (~10^3, 10^1, and 10^{-1} CFU/leaf) were used with spot-inoculated red leaf lettuce serving as a colored surrogate for baby spinach and cilantro. For each of three trials per condition, 5 kg batches of product containing uninoculated baby spinach or cilantro and inoculated red leaf lettuce at each of the aforementioned ratios and inoculation levels was washed for 90 sec in a 3.3-m long flume tank through which 890 L of sanitizer-free tap water was circulated in a pilot-scale leafy green processing line. After washing and removing the red leaf lettuce, all previously uninoculated product (~23 samples/250 g each) was analyzed for presence/absence of *Salmonella* using the *Salmonella* GeneQuence Assay (Neogen Corp. Lansing, MI).

Results: Overall, 100% of the spinach samples washed with red leaf lettuce containing *Salmonella* at 10^3 CFU/g tested positive, regardless of the inoculated:uninoculated ratio. When red leaf lettuce was inoculated at 10^1 CFU/g, 100, 64-81, 52-61 and 12-22% of the samples yielded *Salmonella* at inoculated:uninoculated ratios of 10:100, 5:100, 1:100, and 0.5:100, respectively. At the lowest inoculation level of 10^{-1} CFU/g, 4.3-8.7, 4.3-8.7, 4.3-26.0 and 0% of the samples yielded *Salmonella* at inoculated:uninoculated ratios of 10:100, 5:100, 1:100, and 0.5:100, respectively, with a similar trend seen for cilantro.

Significance: This is the first study to assess the spread of *Salmonella* from incoming product to baby spinach and cilantro during processing. These results provide important data for microbial risk assessments associated with leafy greens.

P2-85 Diversified Farms in California: Can One Tomato Spoil the Barrel?

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Introduction: The number of small- and medium-sized farms that integrate livestock and produce is increasing, but we lack data on how these practices affect produce microbial quality.

Purpose: To evaluate fecal contamination in tomatoes grown on small- or medium-sized diversified farms, and elucidate possible contamination sources.

Methods: Fifteen small- or medium-sized farms located in California (Central Valley=6, Central Coast=5, Shasta Cascade=2, North Coast=2) were sampled from July to October 2015; eight farms were certified organic. A total of 305 tomato samples were collected either randomly or as targeted samples due to animal intrusion or other risk factors. Irrigation water (*n*=78), soil (*n*=113) and soil amendment (*n*= 57) samples were also collected. The Most Probable Number (MPN) of *Escherichia coli* was used as indicator of fecal contamination.

Results: Altogether, 14.29% of samples were positive for *E. coli* (79/553): 4.26% tomatoes, 9.73% soil, 37.18% irrigation water and 45.61% soil amendment. Average MPN values were: 1914 MPN/100 g for tomatoes (range 0 – 460,000), 2,825 MPN/100 g for soil (0- 150,000), 102 MPN/100 ml for irrigation water (range 0 – 4,200) and $11*10^6$ MPN/100 g for soil amendment (0 – $280*10^6$). The source of irrigation water played an important role with an average MPN (range) of: 0 (0-0) in city water, 2.13 (0-92) in ground water, and 327.7 (0.36- 4,200) in surface water used for irrigation. Also, the type of soil amendment had an influence on average MPN and range: 40,770 (0 – $12*10^5$) for finished compost, 116,887 (0 – 720,000) for unfinished compost and $42.47*10^6$ (0 – $280*10^6$) for raw manure.

Significance: The prevalence of *E. coli* in tomatoes from small- and medium-sized farms is low. However, irrigation water and soil amendments (especially raw manure) inputs may be a source of fecal contamination. There is a need for food safety training that addresses produce production on small- and medium-sized farms.

P2-86 Microbial Dynamics of Indicator Organisms on Fresh Tomatoes in the Supply Chain from Mexico to the USA

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Introduction: Quality and safety of fresh produce are important to public health and maintaining commerce between Mexico and USA. While preventative practices can reduce risks of contamination and are generally successful, the variable environment of the fresh produce supply chain can be suitable for introduction or proliferation of pathogenic microorganisms. An opportunity exists to use indicator microorganisms on fresh produce to measure how handling and transport may contribute to their quality or safety.

Purpose: The objective was to quantify microbial indicators on tomatoes sampled along the supply chain, in order to observe the magnitude of population changes due to ambient conditions.

Methods: Roma tomatoes (*n*=475) were taken from the same lots (*n*=28) at four locations of the postharvest supply chain over five months: at arrival to and departure from the packinghouse, at the distribution center and at supermarkets. Samples were analyzed individually for four microbial populations: aerobic mesophiles, total coliforms, generic *Escherichia coli*, and yeasts/molds. Statistical analyses of log CFU/tomato surface were made using generalized linear models (PROC GLM, SAS 9.3) with Tukey-adjusted *P*-values.

Results: Population means and standard deviations for aerobic mesophiles were 2.3±1.1, 1.91±1.1, 2.6±1.1 and 3.8±1.4 log CFU/tomato at postharvest, packing, distribution center and supermarket, respectively. Total coliform populations were below the detection limit at postharvest, but were detectable at packing, distribution and supermarkets at means of 0.86±1.1*, 0.48±0.9*, and 1.6±1.6 log CFU/tomato, respectively. Generic *E. coli* was not detected in this supply chain. Yeast/mold populations remained <1 log CFU/tomato, with the exception of 1.4±1.3 log CFU/tomato at supermarkets, indicative of minimal product spoilage. *Actual minimum is zero.

Significance: The means reported demonstrate the dynamics within populations as influenced by supply chain logistics and conditions, while the large deviation in some locations indicates opportunities for improvement. Overall, packinghouse and supermarket locations were identified as crucial points to control microbial safety risks.

P2-87 *Escherichia coli* Can Internalize into Upper Region of Tomato Stem Scar Channels

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Introduction: Microorganisms on fresh produce, including microbial pathogens, can become internalized within produce, escaping decontamination treatment.

Purpose: This research studied the extent of *Escherichia coli* K12 internalization through the tomato stem scar by post-harvest inoculation without aid of temperature or pressure difference to determine the depth to which internalized organisms may travel within channels in tomato stems.

Methods: Stem-intact vine tomatoes were washed and surface-sanitized using 70% ethanol; samples (*n*=6) were air dried for 1 h. Tomato stems were inoculated with 100 µl 7.0±0.1 log CFU/ml rifampicin-resistant *E. coli* K12. After 1 h attachment period, stems were aseptically removed. Samples were then held at 25°C for 24 h to allow for microbial internalization through stem scars. Afterward, stem scars were excised into 3 pieces with 0.6 cm height each (top, middle, and bottom) relative to the intact stem. Samples were placed in stomacher bags and pummeled with 25 ml 0.1% peptone diluent for 1 min; samples were serially diluted and spread on tryptic soy agar containing 0.1g/liter rifampicin (TSAR). TSAR plates were incubated at 35°C for 24 h and colonies enumerated. Logarithmically-transformed data were analyzed using Student's *t*-test to identify differences in *E. coli* numbers by stem scar depth. Uninoculated stem scar samples were visualized using scanning electron microscopy (SEM) to determine physical characteristics of stem scars.

Results: *E. coli* K12 (2.8 ± 1.6 log CFU/cm^3) were recovered from the top stem scar samples but not the middle and the bottom pieces. The SEM images of internal stem scar samples showed vascular bundle structures with approximately 100 µm length and 15 µm diameter.

Significance: These results suggest deposit of aqueous microbial suspension onto tomato stem could result in internalization through vascular bundles structures, however internalized microbes are unable to travel to cells deep within the stem.

P2-88 Growth and Survival of *Salmonella* spp. on Whole and Sliced Cucumbers

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Introduction: *Salmonella* outbreaks were associated with cucumbers in 2013, 2014 and 2015. The 2014 *Salmonella* outbreak in cucumbers caused 275 illnesses and 1 death in 29 U.S. states and Washington DC. Little is known about the behavior of *Salmonella* on whole and sliced cucumber.

Purpose: A study was performed to evaluate the growth and survival of *Salmonella* spp. on whole and sliced cucumbers at different storage conditions.

Methods: Whole and sliced cucumbers were spot inoculated with a five-strain cocktail of *Salmonella*. Inoculated samples were air dried, placed in Whirl-pak bags and stored at 4 and 24±2°C. Samples were enumerated on nonselective and selective media following storage for 0, 24, 48, 72, 120 and 168 h and 0, 8, 17, 24, 48 and 72 h for cucumbers held at 4 and 24±2°C, respectively. Population levels (log CFU/g) of *Salmonella* on cucumber were calculated.

Results: *Salmonella* populations significantly increased on whole and sliced cucumbers held at room temperature for the storage period (P<0.05). At 24±2°C, *Salmonella* populations increased by approximately 2.5 and 3 log CFU/g on whole and sliced cucumbers, respectively. The majority of the growth occurred during the first 17 h. At 4±2°C, *Salmonella* populations survived for up to 7 d on both whole and sliced cucumber, with an average decrease of 1 log CFU/g.

Significance: Whole and sliced cucumbers are potential vectors for *Salmonella* transmission. Best practices (GAPs, GHPs) should be followed in the field to limit pre-harvest contamination risk, and the cold chain maintained throughout handling to reduce the likelihood of *Salmonella* multiplication.

P2-89 Quantification of *Salmonella* Transfer from Cucumber Skin to Flesh and Peeler during Peeling

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Introduction: Several multistate outbreaks of *Salmonella* linked to cucumbers (*Cucumis sativus* L.) have occurred recently in the United States.

Purpose: Little is known about the degree to which bacteria can transfer from the surface of fresh produce items during peeling. This study quantified factors influencing the transfer of *Salmonella* from the surface of fresh cucumber to interior flesh and peeler during peeling.

Methods: Waxed and un-waxed Garden cucumbers (American slicing) were obtained from a supermarket and local grower, respectively. The effect of a 70% ethanol pre-treatment was also investigated. Wild type *Salmonella* Newport, and putative attachment mutants, JDB 279 (*rpoS*::Tn*10*:lac:kan) and JDB 287 (Tn*10*:lac:kan insertion in the *agfD*/*agfB* intergenic region) were used. Cucumbers were dip inoculated with the designated strain (~10^9 CFU/ml). Samples were dried for 12 or 24 h before peeling. Half of each inoculated cucumber was hand peeled using a sterilized peeler. The unpeeled half, the peeled half (flesh), the removed peel and the peeler were all enumerated for the presence of *Salmonella*. Percent transfer or log reductions were calculated as appropriate. Percent transfer was calculated by recipient surface/donor surface * 100.

Results: The majority (>50%) of *Salmonella* remained on the peel portion for all treatment groups. Relatively greater *Salmonella* transfer to flesh (0.13%) and peeler (0.20%) were observed during peeling of waxed cucumbers washed with 70% EtOH and dried for 24 h compared to other waxed cucumber groups. A significant difference between *Salmonella* Newport wild type and mutant strains (JDB 279 and JDB 287) transfer to the peel portion of waxed cucumbers was noted (P=0.0235). Significantly higher transfer of JDB 287 to flesh (P=0.0187) and peel (P=0.0222) was observed in un-waxed cucumbers prewashed with 70% EtOH.

Significance: This study shows the complex interactions occurring between *Salmonella* strains and cucumber surface characteristics during bacterial transfer during peeling.

Poster

P2-90 Impact of Cutting Speed on *Listeria monocytogenes* Transfer during Slicing of Zucchini Squash and Cucumbers

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❖ ***Developing Scientist Competitor***

Introduction: Increased consumption of fresh-cut produce has led to heighted food safety concerns as evidenced by ongoing recalls and outbreaks.

Purpose: Using cucumbers and zucchini squash as model products based on their inherent compositional differences, this study aimed to evaluate the impact of mechanical slicing speed on *Listeria* transfer.

Methods: Locally obtained zucchini squash and cucumbers were dip-inoculated with a 3-strain avirulent cocktail of *Listeria monocytogenes* to contain ~ 5 log CFU/g, and air-dried for 1 h. After artificially contaminating a modified NEMCO slicer (Model # 59155491) by slicing one inoculated zucchini or cucumber, 15 uninoculated zucchini or cucumbers were cut at a speed of either 3.3 cm/s or 2 cm/s. The first, middle and last slice from each of 15 samples was collected and analyzed for *Listeria* by surface-plating on MOX. Texture was measured using a texture analyzer TA-XT2i and water content was determined using a forced-air oven overnight at 100°C.

Results: After slicing one inoculated sample, the pusher and blades each yielded ~3 log CFU/component. After slicing, the 1st and 15th uninoculated zucchini yielded statistically similar (P < 0.05) *Listeria* populations at the high (3.5±1.1 and 2.7±0.6 log CFU/g) and low (3.4±0.4 and 2.5±0.6 log CFU/g) slicing speeds, respectively. However, for cucumbers significant reductions in *Listeria* transfer (P < 0.05) were observed between the 1st and 15th cucumber at both the high (3.4±0.3 and 2.2±0.4) and low (3.2±0.2 and 2.7±0.2) slicing speeds. Although water content for zucchini (95.6%) and cucumber (94.1%) was similar, the peak positive force for zucchini (11.78 N) was significantly (P < 0.05) lower than for cucumbers (29.43 N).

Significance: The extent of fresh produce cross-contamination is affected by slicing speed. These findings should prove useful in developing improved predictive models for bacterial transfer based on product composition and expanding current risk assessments across a wider range of products.

P2-91 Genetic Characteristics of Isolated *Escherichia coli* from Kimchi Ingredients and Developing a Dynamic Model to Predict *E. coli* Survival in Napa Cabbage Kimchi

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Introduction: *Escherichia coli* can be contaminated in fresh produces such as Napa cabbage which is major ingredient of kimchi, and it may grow or survive during kimchi fermentation.

Purpose: This study investigated the genetic characteristics of *E. coli* isolates in Nappa cabbage and developed a dynamic model to predict the fates of *E. coli* in kimchi.

Methods: Kimchi ingredient samples [Napa cabbage (*n*=30), white radish (*n*=30), leek (*n*=20), ginger (*n*=20)] were analyzed to detect *E. coli*. Isolated colonies were identified by 16s rRNA analysis, and presences of *stx1* and *stx2* were investigated by PCR. O and H types were also determined. To develop a dynamic model, a 5-strain mixture of *E. coli* was inoculated in kimchi at 4 log CFU/g, followed by storage at 4 - 30°C up to 17 days. The Baranyi model was fitted to the microbiological data to calculate maximum specific growth rate (μ_{max}; log CFU/g/h) and death rate (*DR*; log CFU/g/h). Polynomial equations were then fitted to the kinetic parameters, followed by developing a dynamic model. Root mean square error (*RMSE*) was calculated to evaluate the model performance.

Results: Of 30 Napa cabbage samples, one sample was contaminated with *E. coli*, and the *E. coli* was serotyped as O6:H34 with the presence of *stx2* gene. At 4°C and 10°C, *E. coli* survived until 192 h and 132 h, respectively, and then it started to decrease. At 15 - 30°C, *E. coli* in cabbage kimchi grew initially and gradually started to decrease during fermentation. In growth phase, μ_{max} increased as temperatures increased, and *DR* also increased at higher temperatures. The prediction of developed dynamic model was appropriate with 0.586 of *RMSE*.

Significance: The results indicate that *E. coli* from raw ingredients may contaminate kimchi, and the developed dynamic model should be useful in predicting the kinetic behavior of *E. coli* in cabbage kimchi.

P2-92 Migration of *Salmonella enterica*, Artificially Internalized into Vegetable Seeds, to Different Sections of Sprouts/Seedlings during Germination

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Introduction: Vegetable seeds contaminated with *Salmonella enterica* have been linked to fresh produce (especially sprouts)-associated outbreaks of gastrointestinal infections.

Purpose: This study was undertaken to determine if *Salmonella,* artificially internalized into vegetable seeds, could migrate to different sections of sprouts/seedlings during germination process.

Methods: Alfalfa, fenugreek, lettuce and tomato seeds were artificially infiltrated with nalidixic acid-resistant *Salmonella* Montevideo, *Salmonella* Stanley, *Salmonella* Baildon or *Salmonella* Cubana. Contaminated seeds were germinated on 1% water agar in germination boxes at 25°C. Different sections of germinating seeds (whole seed, seed coat, cotyledon, stem, and root) were collected twice daily for 9 days for *Salmonella* enumeration. Each sample had ten replicates per trial, and two independent trials were conducted. Collected samples (*n*=512) were homogenized and aliquots of homogenates were spread-plated in duplicate on bismuth sulfite agar and tryptic soy agar supplemented with nalidixic acid. Obtained data were analyzed by Fisher's LSD test using the R software.

Results: Cells of all four *Salmonella* strains migrated from contaminated vegetable seeds to different sections of the seedlings. Approximately 62% of the 512 samples tested positive for *Salmonella*. The average *Salmonella* population increased from 0.85 to 1.76 log CFU/seed section during seed germination. The highest *Salmonella* count was observed on cotyledons and seed coats (1.97 and 1.89 log CFU/seed section). *Salmonella* Montevideo had the lowest recovery rate of 0.72 log CFU/seed section, in comparison to 1.86, 1.85 and 1.75 log CFU/seed section for *Salmonella* Stanley, *Salmonella* Baildon and *Salmonella* Cubana. *Salmonella* populations recovered from fenugreek and alfalfa sprouts (2.76 and 2.35 log CFU/seed section) were significantly higher (P < 0.05) than those from tomato and lettuce seedlings (0.57 and 0.50 log CFU/seed section).

Poster

Significance: The study revealed the ability of *Salmonella* internalized in vegetable seeds to migrate to different sections of sprouts/seedlings during seed germination, emphasizing the importance of pathogen-free seeds for vegetable sprout production.

P2-93 Migration of *Salmonella enterica* from Inoculated and Accompanying Contaminated Vegetable Seeds to Sprouts or Seedlings

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Introduction: Contaminated vegetable seed is a potential source of foodborne pathogens such as *Salmonella enterica.*

Purpose: This study was undertaken to determine whether *Salmonella* 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 different nalidixic acid-resistant strains of *Salmonella* (B, C, M, S) by artificial inoculation (IN) or accompanying contamination (AC). For IN, overnight *Salmonella* culture was co-incubated with the vegetable seeds at 20°C for 5 h, whereas for AC, freeze-dried *Salmonella* cells in sterile sandy soil were co-incubated with the 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 *Salmonella* on different tissues (seed coat, cotyledon, stem and radicle) of the sprouts/seedlings were determined every other day for 5 days. Data was analyzed by Fisher's LSD using SAS software.

Results: Approximately 94% and 64% of the samples inoculated by IN and AC, respectively, tested positive for *Salmonella*. For both inoculation methods, seed coats had the highest *Salmonella* level (5.35 log for IN and 3.59 log for AC), followed by radicle (5.12 log for IN and 3.40 log for AC), cotyledon (5.00 log for IN and 3.25 for AC) and stem (4.50 log for IN and 2.92 for AC). Artificially inoculated fenugreek seeds had the highest *Salmonella* level (5.81 log), followed by alfalfa (5.67 log), lettuce (5.48 log) and tomato (1.54 log) seeds. For AC, lettuce seeds had a higher *Salmonella* level (3.80 log) than alfalfa seeds (2.26 log). For both inoculation methods, strain M had the lowest rate of recovery.

Significance: These data suggest that *Salmonella* migrated from artificially inoculated and accompanying contaminated vegetable seeds to various tissues of sprouts/seedlings during germination, indicating the importance of using pathogen-free seeds for vegetable sprout/seedling production.

P2-94 *Listeria* Inter-species Competition during the Selective Enrichment of Spiked Mung Bean Sprouts

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Introduction: *Listeria monocytogenes* can be difficult to recover following selective enrichment when non-pathogenic *Listeria* species are concomitantly present in the test sample. Naturally high microbial levels, in foods such as mung bean sprouts, can further complicate recovery because of variation in the growth rate under selective conditions.

Purpose: This study establishes the final population densities of select strains of *L. monocytogenes* and *L. innocua* following selective enrichment of spiked mung bean sprouts. Additionally, this study estimates the inter-species population differential (Δlog CFU/ml) that results when two *Listeria* species are present together during the enrichment.

Methods: Portions of mung bean sprouts were spiked (1 to 5 CFU/g) with a *L. monocytogenes* strain or a *L. innocua* strain or both together. Ten strains of each species were studied. Selective enrichment was performed using the FDA-BAM method. PALCAM agar was used to estimate the *Listeria* populations of single-species spiked sprout enrichments. qPCR was used to enumerate *L. monocytogenes* in the double-spiked enrichments.

Results: Substantial *L. monocytogenes* population suppression (≈ 3 Δlog CFU/mL) and large *Listeria* inter-species population differentials (≈ 3 Δlog CFU/ml) were observed. The mean post-enrichment *L. monocytogenes* population of singly-spiked sprouts was 6.1±1.2 log CFU/ml. In matrix-free enrichments, the mean population was 9.3±0.2 log CFU/ml. The mean post-enrichment populations of doubly-spiked sprouts were 4.7±1.1 and 7.6±0.2 log CFU/ml, for *L. monocytogenes* and *L. innocua*, respectively.

Significance: Detection of *L. monocytogenes* and recovery of the organism from high microbial load food products may be hindered as a result of microbial competition. Continued research toward the improvement of *L. monocytogenes* enrichment methods is needed to enhance the capabilities of regulatory agencies to detect and recover *L. monocytogenes* amidst a complex microflora.

P2-95 Transcriptomic Analysis of *Listeria monocytogenes* Grown on Refrigerated Cantaloupe Slices

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Introduction: The foodborne pathogen *Listeria monocytogenes* has been associated with a variety of foods. Prior to a successful foodborne transmission to a human host, the pathogen has to adapt in a specific food through modulation of its gene expression in order to ensure its survival and growth in foods. Understanding of the pathogen's response to diverse food environments is imperative to devise effective control strategies as well as elucidate potential impact of foods on virulence potential.

Purpose: The purpose of this study was to assess transcriptional response of *L. monocytogenes* during growth in refrigerated cantaloupe.

Methods: The transcriptional profiles of *L. monocytogenes* grown to stationary phase in cantaloupe slices or brain heart infusion (BHI) under refrigeration were compared by a custom-designed microarray. The microarray was designed to represent 16,724 annotated gene sequences from 41 closed chromosomes of *L.* monocytogenes, 5 plasmids, and 16 whole genome shotgun sequences which were available from GenBank as of April, 2014.

Results: A total of 216 and 181 genes were significantly up- and downregulated in *L. monocytogenes* grown in cantaloupe as compared to BHI (fold change ≥ 2.5 and FDR < 0.05). Majority of the upregulated genes belonged to functions related to amino acid and nucleotide transport and metabolism while the majority of downregulated genes belonged to carbohydrate transport and metabolism. Interestingly, transcript levels of several stress- and virulence-related genes were significantly altered, implying the potential impact of growth in cantaloupe slices on stress resistance and virulence potential of *L. monocytogenes*.

Significance: Evaluation of transcriptional landscape of *L. monocytogenes* during growth in foods such as cantaloupe is an effective approach to determining how the pathogen adapts and grows in complex food systems. The resulting knowledge has the potential to facilitate development of novel control strategies as well as improved risk assessment.

Poster

P2-96 Enumeration of *Listeria monocytogenes* Contaminated Ice Cream Products Distributed to Public Commerce

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Introduction: Listeriosis is an invasive illness typically caused by the ingestion of food products contaminated with *L. monocytogenes.* An outbreak of listeriosis in 2015 was linked to ice cream produced at plants in two different states linked to the same firm. One of the implicated products, Product C, was produced on a specific production line at one plant. The FDA was able to obtain and enumerate samples representing several lots of three of the Products, A, B and C, made on that line. Product C, linked to illness, was evaluated separately.

Purpose: Two products, A and B, not linked to illness, were enumerated in this study via a most probable number (MPN) method to determine their contamination level for future risk assessment and dose-response analyses.

Methods: An MPN scheme with a detection limit of 0.03 MPN/g was used to analyze 344 samples of Product A from ten lots and 95 samples of Product B, representing five lots. These products encompass production dates spanning a nine-month period.

Results: A total of 76% of Product A samples were positive for *L. monocytogenes* while 46% of Product B were positive. Additionally, the contamination level was less in Product B than Product A (median of 0.02 MPN/g and 0.1 MPN/g, respectively). All positive Product B samples had *L. monocytogenes* at levels of less than 1 MPN/g. Product A contamination levels were predominantly less than 1 MPN/g (52%) with the remaining 24% mostly between 1 to 50 MPN/g, with only one sample above 100 MPN/g.

Significance: These enumeration data show the extent and variability in contamination levels of *L. monocytogenes* in these ice cream products manufactured on a contaminated line. These data, combined with the epidemiological data, will improve our understanding of the contamination patterns, as well as dose response and risk assessment.

P2-97 Survival of *Listeria monocytogenes* on 'Athena' and 'Rocky Ford' Cantaloupes Stored at 4°C, 10°C, and 25°C

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Introduction: Whole cantaloupes (*Cucumis melo* L), marketed as 'Rocky Ford,' were implicated in a large United States multi-state outbreak of listeriosis in 2011.

Purpose: The role of cultivar, storage temperature, and site of contamination on the survival and/or growth of *Listeria monocytogenes* on whole cantaloupes was evaluated.

Methods: 'Athena' and 'Rocky Ford' cantaloupe cultivars were grown in fields or high tunnels and harvested at half- to full-slip, and stored at 4°C until inoculated. A multi-strain inoculum of *L. monocytogenes* (6 log CFU/cantaloupe) was spot-inoculated on the rinds or stem-scars of individual melons, and subsequently stored at 4, 10, and 25°C. *L. monocytogenes* populations on individual cantaloupes at each temperature were determined for up to 15 days. Also, *L. monocytogenes* was inoculated into rind extracts, and *L. monocytogenes* populations and aerobic populations of rind extracts determined during storage at 4°C and 25°C for 7 days. A linear model for ANOVA using a factorial mode was used to compare mean bacterial populations.

Results: *L. monocytogenes* populations on stem-scars of whole cantaloupes stored at 25°C increased by approximately 2 log CFU after one day, and were significantly greater (P<0.05) than those on whole cantaloupes stored at 4°C and 10°C, which did not increase in the same time-frame. *L. monocytogenes* populations decreased on cantaloupe rinds by 2-4 log CFU after 7 days of storage at all three selected temperatures, and were not significantly different (P>0.05). In rind extracts stored at 25°C, populations of aerobic bacteria increased while those of *L. monocytogenes* decreased at day 3; however, at 4°C, growth of *L. monocytogenes* populations were greater than aerobic populations at 7 days. There were no significant differences in *L. monocytogenes* populations based on cultivar.

Significance: Site of contamination and storage temperature influenced growth and survival of *L. monocytogenes* on whole cantaloupe surfaces. *L. monocytogenes* can proliferate on stem-scars of whole cantaloupes.

P2-98 Growth of *Listeria monocytogenes* on Fresh-cut Pieces of Cantaloupe from Two Different Varieties during Storage

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Introduction: 'Rocky Ford' cantaloupes were implicated in the U.S. multi-state outbreak of listeriosis in 2011. *Listeria monocytogenes* can survive on whole cantaloupes during storage, and may be transferred to flesh of melons.

Purpose: The growth of *L. monocytogenes* on fresh-cut 'Athena' and 'Rocky Ford' cantaloupe cultivars during refrigerated storage was evaluated.

Methods: Fresh-cut cubes (1 in^3) of cantaloupes inoculated with a multi-strain inoculum of *L. monocytogenes* (5 log CFU/cube) were stored at 4°C and 10°C for up to 7 days. Also, inoculated fresh-cut cubes were continuously stored at 4°C for 3 days or stored at 4°C and subjected to temperature abuse (TA – 25°C/4 h) events on days 0 and 1, and then stored at 4°C until day 3. *L. monocytogenes* populations were determined on selected days and after each TA event. Brix values were measured for both cantaloupe cultivars. An ANOVA was performed to determine storage temperature and cultivar effects on *L. monocytogenes* populations on fresh-cut cubes.

Results: *L. monocytogenes* populations on fresh-cut cantaloupes stored at 4°C increased by approximately 1 log CFU/cube by day 7, whereas populations increased by approximately 3.5 log CFU/cube on those stored at 10°C by day 7. *L. monocytogenes* populations on fresh-cut cantaloupes stored at 10°C were significantly (P<0.05) greater than those stored at 4°C. Growth of *L. monocytogenes* on 'Athena' and 'Rocky Ford' fresh-cut cantaloupes were similar, although 'Athena' had significantly (P<0.05) greater Brix values than 'Rocky Ford' cantaloupes. *L. monocytogenes* populations on fresh-cut cantaloupes subjected to TA were approximately 0.74 log CFU greater than those that were continuously stored at 4°C at day 3.

Significance: *L. monocytogenes* populations grew on fresh-cut cantaloupes stored at both 4°C and 10°C, and temperature abuse events promoted growth of *L. monocytogenes* on fresh-cut cantaloupe cubes, even when stored at 4°C.

P2-99 Food Safety Risks with Watermelons Grown Using Poultry Litter

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Poster

Introduction: Poultry litter is a widely used biological soil amendment. While research shows that bacterial pathogens exhibit enhanced survival in poultry litter; risks associated with watermelons grown in soils amended with poultry litter are not well understood.

Purpose: This study evaluated the survival of *E. coli* and naturally occurring *Salmonella* in field-grown watermelons in unamended soils and soil amended with poultry litter (PL).

Methods: Two trials of 12 individual plots were sampled from May-November 2015. Seedless Exclamation and Liberty watermelons were planted on plastic mulch in soil or in soil amended with one of two types of PL (cake and total clean out) applied at 6725 kg/Ha. In each trial, one half of the plots were inoculated with non-pathogenic *E. coli* (TVS 355). Soil samples collected weekly (*n*=1032) and watermelons collected at harvest (*n*=120 on days 70, 77, and 84) were analyzed for *E. coli* populations by colony count or MPN, and for *Salmonella* by enrichment using a modified FDA BAM procedure. Data were analyzed using two-way ANOVA in JMP Pro-11.

Results: Composite soil samples and weather parameters were collected weekly. On day 2, *E.coli* levels were significantly ($P<0.0001$) greater in amended soils (7.04 log CFU/g) compared to unamended (4.94 log CFU/g), and remained that way throughout the study. Type of PL did not affect *E. coli* populations. By day 133, *E. coli* populations in PL-amended soils were significantly greater (0.51±1.15 log MPN/g) compared to those in unamended plots which were below the detection limit (-0.6 log MPN/g). *Salmonella* spp. was detected in 36% of soil samples and with increased frequency during July-August ($P<0.05$). Over the study, more samples taken adjacent to the plastic mulch were positive for *Salmonella* compared to samples from under mulch ($P>0.05$). Approximately 50% of watermelons sampled were positive for *E.coli* with a highest recovery of 2.14 log (MPN/g) from amended-soils. *Salmonella* spp. was detected on watermelons (16/120) grown in both amended and unamended soils.

Significance: Incorporation of poultry litter provided conditions conducive to survival of *E. coli* and introduced *Salmonella* into soil and on watermelons.

P2-100 Determination of Growth Potential of *Salmonella* and *Listeria monocytogenes* in the Pulp of Eight Exotic Fruits

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Introduction: In recent years, an increase in the number of outbreaks associated with consumption of fresh fruit has been reported. There are no published data on the fate of *Salmonella* and *Listeria monocytogenes* in several exotic fruits.

Purpose: The purpose of this study was to determine the growth potential (δ) of *Salmonella* spp. and *L. monocytogenes* in the pulps of exotic fruits stored at 10, 20, 30 and 37°C.

Methods: Pulp samples of jenipapo, umbu, maná, cajá-manga, physalis, feijoa, cupuaçu and abiu were separately inoculated with a cocktail of three and two strains of *Salmonella* spp. and *L. monocytogenes*. High-acid and low-acid fruits were inoculated with 10^6 CFU/g and 10^3 CFU/g of cocktails of each microorganism, respectively. After inoculation and homogenization, pulps were stored at 10, 20, 30 and 37°C, and counts of *Salmonella* and *L. monocytogenes* were done in MLCB and Oxford agars in 0, 2, 4, 6, 8, 10 and 12 h. The δ was determined by the difference between the counts at the end (12 h) (log CFU/g) and at the beginning (time "zero") (log CFU/g) of storage period.

Results: Both *Salmonella* and *L. monocytogenes* were able to grow in abiu pulp (pH> 6.10) at 10-37°C. These pathogens lost viability in umbu and jenipapo pulps (3.7-6.2 log reductions within 2 h, respectively) regardless of storage temperature. In mana, cajá-manga and cupuassu these pathogens lost viability at 30 and 37 °C/2-8 h, but survived at 10 and 20°C during 8-12 h. In cupuassu, *Salmonella* and *L. monocytogenes* were inactivated after 8 h at 20°C while in physalis and feijoa they survived for 12 h in all storage conditions.

Significance: The knowledge on the δ of pathogens in exotic fruits is important to allow measures to be taken to safeguard public health.

P2-101 Growth of *Salmonella* spp. in the Peel and in the Pulp of Avocado (*Persea americana*)

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Introduction: The occurrence of cases and outbreaks of foodborne illness associated with the consumption of fruit have been reported in the last years. Data from epidemiological investigations indicate that *Salmonella* is the main etiological agent associated with foodborne disease outbreaks involving fruits. Avocado is a tropical fruit produced in Brazil, whose consumption and exports have increased in recent years. Despite this, no data on the behavior and on the growth kinetics parameters of *Salmonella* in this fruit are found in the literature. This fact is of great concern, since it is known that the low acid fruits constitute optimal substrates for the multiplication of microorganisms, especially pathogens.

Purpose: To determine the growth kinetic parameters (maximum growth rate, μ; lag time; λ and maximum population, κ) of *Salmonella* spp. on the peel and in the pulp of avocado at 10, 15, 20 and 30°C.

Methods: Samples of peel and pulp of avocado were inoculated with a cocktail of three strains of *Salmonella* spp. (*Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Montevideo) (10^2 to 10^3 CFU/g) and further stored at 10, 15, 20 and 30°C. Sampling was carried out at different intervals and count was done using MLCB agar incubated at 37°C/ 24 h. The growth kinetic parameters were obtained by fitting the experimental data to Baranyi model using the DMFit software. The growth kinetic parameters of *S. enterica* were checked for significant statistical differences ($P\leq0.05$) employing one-factor analysis of variances (ANOVA) followed by Skott-Knott test.

Results: The growth kinetic parameters indicated that *Salmonella* was able to grow on the peel and pulp of avocado from 10 to 30°C, presenting significant different λ and κ at 30°C ($P<0.05$). μ does not seem be influenced by substrate of growth (pulp or peel).

Significance: The results showed that avocado comprise substrates that allow the growth of pathogens such as *Salmonella*, both in the pulp and also in the peel, offering risk to public health.

P2-102 Prevalence and Populations of *Listeria monocytogenes* and *Salmonella* spp. in Brazilian Artisanal Cheeses

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Introduction: Artisanal cheeses (AC) have strong historical and social connections with the communities in which they are produced. AC are produced from raw milk and highly consumed in some regions of Brazil.

Purpose: This study aimed to determine the incidence and concentration of *Listeria monocytogenes* and *Salmonella* in Brazilian AC.

Methods: We analyzed 582 samples of Brazilian AC, divided according to their production region: North (Marajó; *n*=8); Northeast (Curd cheese; *n*=78) and (Butter cheese; *n*=23); Central (Caipira cheese; *n*=108); Southeast (Araxá; *n* = 56), (Campo das Vertentes; *n* = 54), (Cerrado; n = 54), (Serra da Canastra; *n* = 48) and (Serro; *n* = 50) and South cheeses (Colonial; *n* = 55) and (Serrano; *n* = 48). Detection and enumeration of *L. monocytogenes* was performed according to ISO 11290-1 and ISO 11290-2. Identification of *L. monocytogenes* was performed by biochemical tests and real-time PCR (RT-PCR) with amplification of listeriolysin gene (*hlyA*). Detection of *Salmonella* based on ISO 6579: 2002, while for enumeration ISO 6579-2: 2012 was used.

Results: Presumptive colonies of *L. monocytogenes* were found in 11% of samples. From 266 presumptive colonies of *L. monocytogenes*, seven were recovered from Butter (*n*=5 isolates), Serro (*n*=1 isolate) and Curd (*n*=1 isolate) cheeses and confirmed by RT-PCR. *Salmonella* was not detected in any of the 582 AC samples analyzed. Populations of *L. monocytogenes* and *Salmonella* were always below the limit of quantification of methods used (1 log CFU/g).

Significance: The prevalence of *L. monocytogenes* was very low (0.005), while *Salmonella* was not detected in any of 582 AC samples analyzed, indicating that practices used to produce them are adequate from hygienic point of view.

P2-103 Incidence and Characterization of *L. monocytogenes* in the Stone Fruit Production Continuum

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Introduction: Recent recalls due to contamination by *Listeria monocytogenes* and listeriosis outbreaks due to consumption of caramel apples and stone fruits indicate a need for a better understating of the incidence behavior of this pathogen in the fruit production continuum.

Purpose: To obtain environmental surveillance data on the incidence and prevalence of *L. monocytogenes* on stone and pome fruits in pre-harvest and post-harvest fruit production environments.

Methods: Stone fruit collection in the orchards and environmental sampling at the Penn State Fruit Research and Extension Center stone fruit packing and storage facility was conducted during 2014 and 2015 seasons. *L. monocytogenes* detection and identification was conducted following BAM protocols. Whole genome sequencing of *6 L. monocytogenes* isolates was completed via Illumina platform. Multi locus sequence (MLST) and core genome MLST analyses were performed to compare these isolates with other fully sequenced *L. monocytogenes* strains.

Results: Overall, 15.3 % (*n* = 216) of the postharvest environmental samples were positive for *Listeria*; however, no *L. monocytogenes* was found in the fruit packing and storage facility. The incidence of the *L. monocytogenes* on intact stone fruits in the orchards was 1.1% (*n* = 540). MLST analysis identified 3 isolates belonging to sequence type (ST) 368 and 3 other belonging to a novel ST. Core genome MLST identified the isolates with ST368 closely related to a strain from natural environment (sidewalk) with 12 allele differences. The isolates with the novel ST were not closely related to any of the fully sequenced strains and the closest match was a strain from an animal source with 556 allele differences.

Significance: Identification of the biological and environmental factors leading to stone fruit contamination by *L. monocytogenes* will facilitate the development of preventive control strategies to significantly reduce the number of recalls and foodborne listeriosis associated with consumption of stone fruits.

P2-104 Survival of Generic *E. coli* on Apples with Overhead Evaporative Cooling Treatment Prior to Harvest

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Introduction: Overhead evaporative cooling (EC) using surface water is frequently used in Washington to decrease sunburn in apples to prevent economic losses, but its influence on food safety risk is uncertain.

Purpose: Reduction of inoculated generic *E. coli* levels was observed on apples varieties with and without EC water application for up to two weeks.

Methods: A four-strain, rifampicin-resistant generic *E. coli* cocktail was inoculated onto apples of selected varieties (Gala and Golden Delicious) with or without EC and harvested from three canopy positions (high, low outside, low inside). For examination of survival on mature fruit near harvest (short-term) varieties were sampled at 0, 2, 10, 18, 34, 42, 58, 82, 106, and 154 hours, for examination of longer-term survival on immature fruit, apples were collected at: 0, 2, 8, 32, (56 or 80), 104, 152, and 320 hours post-inoculation.

Results: Initial inoculum levels on apples ranged from 6.8-7.7 log CFU generic *E. coli*/apple. Rapid die-off of 0.7-3.8 log CFU/apple was observed for generic *E. coli* in the first 8-10 hours, between sun-set and sunrise. For the short-term study, Gala apples at 10 hours, untreated, average reduction in generic *E. coli* was over 2.5 log CFU/apple and for treated Gala over 3.5 log CFU/apple. For the long-term study, untreated Gala apples averaged a 3.7 log reduction of generic *E. coli* during the first 8 hours post-inoculation and the EC treated Gala apples averaged a 3.8 log reduction.

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.

P2-105 *Listeria* Risk Assessment of Apple Packing Facilities

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Introduction: Recalls have highlighted *Listeria* risk in tree fruit packing environments.

Purpose: The effectiveness of sanitation methods and microbial prevalence on apples were evaluated to assess potential risks.

Methods: *Listeria* spp. samples were collected from cold storage rooms and outdoor areas; a comparison of sanitation practices was performed. Samples were analyzed and compared using several methods for generic *Listeria*, and in some cases, *Listeria monocytogenes*. For examination during storage, apples with and without direct surface water contact were harvested, held under refrigerated atmosphere (RA) storage (*n*=200) or controlled atmosphere (CA) storage with ozone (*n*=218) and without ozone (*n*=198) treatment and tested for total coliforms, generic *E. coli*, *Listeria* spp., *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp.

Results: Before cleaning, generic *Listeria* was detected in storage rooms. Only aggressive cleaning vastly reduced observation of *Listeria* spp. in all areas examined, including damaged floors and floor bumpers. Generic *Listeria* were observed on forklifts, cleaning equipment and outdoor traffic areas. Examination of methods showed variability among methods, however 6 of 9 areas examined (equipment, non-line equipment, line support, drain, drencher parts, and cold room floors) were confirmed positive by qPCR or IMS followed by qPCR. Apples directly contacted by surface irrigation water had a higher presence of generic *E. coli* (12.3%; 19/155) compared to controls (3.3%; 6/181). Presence of total coliform and generic *E. coli* decreased during storage; ozone treatment may have reduced microbial levels during CA storage.

Significance: Microbial contamination can enter packinghouses on fruit or through routes of contamination associated with vehicles, cleaning equipment and worker foot traffic. Emphasis on cleaning and sanitizing in production areas is necessary to prevent *Listeria* contamination.

Poster

Poster

P2-106 Investigating the Use of *Bacillus subtilis* as a Biocontrol Agent for *Listeria monocytogenes* on Caramel Apples and Stainless Steel Surfaces

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❖ ***Developing Scientist Competitor***

Introduction: *L. monocytogenes* persists in diverse environments including food matrices and production surfaces. In 2014, *L. monocytogenes* contamination of caramel apples resulted in 7 deaths and 35 illnesses. Unique control measures are needed to reduce the risk of *L. monocytogenes* contamination, like *Bacillus subtilis* UD1022 which has been shown to inhibit the growth of *L. monocytogenes* in culture.

Purpose: The purpose of this project is to evaluate the use of a plant growth promoting rhizobacteria, *Bacillus subtilis* UD1022, as a biocontrol agent to reduce the risk of contamination by *L. monocytogenes* on caramel apples and stainless steel surfaces.

Methods: Stainless steel coupons were inoculated with UD1022 (5.08±1.18 log CFU/ml) in 10% LB broth solution and incubated for 48 h at 22°C. The coupons were rinsed with sterile water and placed into media inoculated with *L. monocytogenes* (8.19±0.08 log CFU/ml). After 48 h, coupons were rinsed and then placed in BPW with glass beads and vortexed for 1 min. Apples were co-inoculated at the stem scar with 4.30-4.60 log CFU *L. monocytogenes* and *UD1022* or *Pseudomonas fluorescens,* air-dried, prepped by stick insertion and then dipped in caramel (85-90°C). Following incubation at 22°C for 0 – 240 h, the apples were placed in 1% BPW, hand massaged for 3min, and bacteria were enumerated on Brilliant Listeria Agar (*n*=6).

Results: Recovery of *L. monocytogenes* from stainless steel coupons was 1.20 ± 0.86 log lower when treated with UD1022 compared to *L. monocytogenes* only controls, significant (*P*=0.0423). Within the first 24 h, no *L. monocytogenes* growth was observed on caramel apples co-inoculated with UD1022, apples with *L. monocytogenes* alone had 2.18 log CFU/ml recovered. By day 5 post-inoculation, *L. monocytogenes* levels were similar in all samples (4.36-4.96 log CFU/ml), including apples that were co-inoculated with *P. fluorescens*.

Significance: UD1022 may be of use as a natural biocontrol agent to reduce the risk of contamination of apple and production surfaces by *L. monocytogenes*.

P2-107 Survival and Growth of *Listeria monocytogenes* during Production and Storage of Caramel Apples

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❖ ***Developing Scientist Competitor***

Introduction: During the fall of 2014, commercially produced pre-packaged caramel apples were linked to 35 cases of listeriosis in 12 states.

Purpose: Consequently, this study aimed to assess 1) the reduction of different outbreak and non-outbreak strains of *Listeria monocytogenes* on caramel apples after dipping and 2) subsequent growth of the apple outbreak strains in caramel apples at 22 and 4°C.

Methods: In aim 1, three Jonathan apples were dip-inoculated with three different 3-strain *L. monocytogenes* cocktails (apple outbreak, unrelated outbreak or unrelated environmental) at ~8 log CFU/apple and then then dipped for 10 s in caramel at 82, 88, 93 or 99°C. One hour later, the apples were massaged in buffer and plated for survivors. In aim 2, Jonathan apples were spot-inoculated at the stem juncture with the apple outbreak cocktail at 3 log CFU/apple, dried for 1 hour, pushed onto wooden sticks, and dipped in caramel at 82°C. At various times during storage at 22 or 4°C, four different apple sections (top, middle, bottom, core) were cut from three apples homogenized and plated for *Listeria*.

Results: After dipping apples in caramel at 82 and 99°C, the apple outbreak, unrelated outbreak and environmental *Listeria* strains decreased 2.0 and 2.7, 1.8 and 2.6, and 1.7 and 2.9 logs, respectively, with the environmental cocktail significantly less heat resistant ($P < 0.05$) at 99˚C than the other two cocktails. After 14 days of storage at 22°C, *Listeria* populations were significantly higher ($P < 0.05$) in the core (7.4 + 0.2 log CFU/g) compared to the other three sections (4.9 – 5.4 log CFU/g). The same trend was seen for the core (7.7 + 0.1) and other three sections (5.0 – 5.4 log CFU/g) after 28 days of storage at 4°C.

Significance: Since dipping in hot caramel will not eliminate *Listeria*, caramel apples should be refrigerated to minimize pathogen growth.

P2-108 Fate of *Listeria monocytogenes* in Caramel Apples Made with Potassium Sorbate-treated Sticks

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Introduction: An outbreak of listeriosis in late 2014 and early 2015 associated with caramel apples brings into question how they became a vector for *Listeria monocytogenes*. Caramel apples were not considered a risk due to the low pH of apples and the low water activity of caramel. Previous work, however, has determined that stem end-inoculated caramel apples with sticks provide an adequate environment for *L. monocytogenes* proliferation. More work is needed to understand effective controls for the pathogen in caramel apples.

Purpose: To determine if sticks pre-treated with potassium sorbate controlled the proliferation of *L. monocytogenes* on stem-end inoculated caramel apples during storage.

Methods: Granny Smith apples were stem end-inoculated with 7 log CFU *L. monocytogenes* per apple, using a cocktail comprised of 3 strains isolated from the caramel apple outbreak, and dried for 2 h. Wood sticks were soaked in 0, 20, 30, 40, or 50% potassium sorbate overnight and dried prior to insertion into the stem end of the apples. Apples were caramel-dipped, dried and packaged into clam-shells. Caramel apples were stored at 25°C for 13 days, and at various timepoints, homogenized with BLEB. Serial dilutions were plated onto PALCAM for enumeration.

Results: Prior to packaging, the *L. monocytogenes* population in caramel apples made with sticks treated with 50% potassium sorbate was significantly lower (4.33±0.91 log CFU/apple, *P*<0.05) than all other conditions (approximately 6 log CFU/apple). Populations were also significantly lower in caramel apples made with sticks treated with both 40 and 50% potassium sorbate after 1 and 2 days; however, populations for all treatment conditions increased to 9 log CFU/apple after 6 days. No differences were seen when comparing 0, 20, and 30% treatments at any timepoint.

Significance: This study will help to determine possible stick pre-treatment conditions to diminish *L. monocytogenes* growth on caramel apples.

P2-109 Concentration, Extraction, and Detection of Enteric Viruses in Raspberries and Blackberries

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Introduction: Norovirus (NoV) and hepatitis A virus (HAV) are recognized as leading causes of foodborne illnesses. Recently, these enteric viruses have been implicated in viral gastrointestinal illness associated with soft fruit. Advancements in research have led to the development of rapid and sensitive methods for the extraction and detection of NoV and HAV in soft fruit.

Purpose: The objective of this research was to develop a sensitive and specific method for the concentration, extraction, and detection of enteric viruses in raspberries and blackberries.

Methods: An ultracentrifugation protocol, with the inclusion of murine norovirus (MNV) as an extraction control, was used to concentrate HAV and MNV in fresh and frozen raspberries and blackberries. The berries were spiked with 22, 2.2, and 0.2 PFU/g of HAV and were spiked at 100 PFU/g of MNV. A QiaShredder and the QiaAMP Viral RNA kit were used for RNA extraction followed by RT-qPCR detection.

Results: HAV was detected in fresh and frozen raspberries and blackberries at 22, 2.2, 0.2 PFU/g. The average extraction efficiency of MNV was 50% with minimal to no inhibition. Inhibition was determined by comparing the IAC Ct value of the RT-qPCR negative control and the IAC Ct value of the sample where minimal inhibition is three Ct values or fewer.

Significance: A fast and reliable method for the extraction and detection of enteric viruses in foodborne associated outbreaks is an integral part of outbreak investigation. Overcoming the acidity, pectin, and polyphenols of soft fruit, particularly in raspberries and blackberries, has led to the development of a viable method for detection of HAV at low levels.

P2-110 Microbial Quality of Blueberries for the Fresh Market

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❖ ***Developing Scientist Competitor***

Introduction: Harvested blueberries are routinely sorted in packing house to remove pre-mature fruits and plant debris, packaged in plastic clam-shells, and cooled in refrigeration facilities before being shipped to the fresh markets. Fresh berry packing involves no kill steps and berry consumption has been linked to foodborne outbreaks of infections.

Purpose: This study determined the microbial quality of fresh blueberries collected from berry-packing houses.

Methods: Unpacked and packed blueberries (*n*=92) were collected in duplicate in the morning, lunchtime, and evening from five different packing house in Georgia from May to June of 2015. Berries were rinsed (n=92) or homogenized (*n*=92) in 0.1M PBS and appropriate dilutions were inoculated on tryptic soy agar, MacConkey agar, Enterococcus agar and potato dextrose agar and resulting microbial colonies were enumerated. The influence of packing, sample source, sampling time, and sample preparation method on berry-borne microbial counts was determined.

Results: Average aerobic bacteria, yeast and mold, and total coliform counts of collected berries ranged from 3.13 to 5.20, 3.76 to 4.85, and 0.62 to 2.21 log CFU/g, respectively. Six berry samples from two processing plants tested positive for fecal coliforms and one sample tested positive for enterococci. Sample source and sampling time have a significant influence ($P \leq 0.05$) on the aerobic bacteria counts, and yeast and mold counts. Berry samples collected in the evening had the lowest microbial counts. Packing of fresh berries had no significant influence ($P > 0.05$) on microbial counts, however, non-packed berries had higher aerobic bacteria, yeasts and molds counts than packed berries. Homogenization of berries before enumeration resulted in higher yeast and mold counts than rinsing.

Significance: This study suggests that sorting of blueberries before packing for the fresh market does not lead to reduction in microbial counts. Packed blueberries are labelled as not ready to eat products therefore, thorough washing of retail blueberries before consumption should be encouraged.

P2-111 Hygiene Conditions of Fresh Blueberry Packing Lines

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Introduction: Foodborne outbreaks have been linked to the consumption of blueberries contaminated with pathogenic bacteria. The microbiological quality of blueberries is strongly influenced by the conditions of hygiene prevailing during their processing and handling.

Purpose: This study is undertaken to evaluate the hygiene conditions of specific sites along fresh blueberry packing lines in Georgia.

Methods: Five fresh blueberry packing establishments in Georgia were selected. A delimited area (100 cm^2) on ten selected sites in each establishment was swabbed with sterile sponges in the morning, at lunchtime and in the evening. The sponges were thoroughly rinsed with Dey-Engley neutralization broth, and the broth were subsequently sampled for total aerobic bacteria, yeasts and molds, total coliforms, fecal coliforms and enterococci.

Results: Average aerobic bacteria counts, yeast and mold counts, and total coliform counts on the selected sampling sites ranged from 2.34 to 3.74, 1.90 to 3.36, and 0.60 to 1.31 log CFU/cm^2, respectively. Sample source, sample site, and sampling time had a significant influence ($P \leq 0.05$) on total aerobic bacteria, yeasts and molds and total coliform counts. The evening samples had the highest microbial counts whilst the morning samples had the lowest counts. Thirty-two out of the 230 (13.9%) sampled sites tested positive for enterococci whilst 20 sites (8.7%) tested positive for fecal coliforms. Seven sites sampled in the morning and evening tested positive for fecal coliforms whereas 6 sites sampled at lunchtime tested positive. Five, 16 and 11 samples collected in the morning, lunchtime and evening, respectively tested positive for enterococci. Berry lugs, rubber belts on color sorters, and pre-mature berry disposing areas had relatively higher microbial counts.

Significance: The study suggests that some sites along the packing lines could be potential sources of blueberry contamination during processing. Sanitation and cleaning practice of blueberry packing houses need to be improved to minimize contamination of freshly packed blueberries.

P2-112 Survival of *Salmonella* during Storage on Three Different Tree Nut Varieties at Three Temperatures and Two Different Relative Humidity Levels

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Introduction: *Salmonella* has been documented to survive for extended periods of time on almonds and pecans, but extrinsic factors such as temperature, and humidity of storage have not been thoroughly examined.

Purpose: This study was conducted to examine the effect of temperature and humidity of storage on the survival of *Salmonella* on three different varieties of tree nuts.

Methods: Three different tree nut varieties - pecans, hazel nuts and pine nuts - were chosen for examination based on differences in their typical fat content. Five different serotypes of *Salmonella* were grown to stationary phase on trypticase soy agar supplemented with 0.6% yeast extract and harvested for use as inoculum for each tree nut variety. Inoculated nuts were stored at 4, 10, and 25°C at two different humidity levels (57% RH and 34% RH). Each nut variety at each storage condition was monitored for one year by periodically removing 10-g quantities for testing for surviving *Salmonella* populations (plating on selective and non-selective media) and water activity.

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Results: *Salmonella* populations were reasonably stable at both humidity levels when stored at 4 and 10°C with a less than 1.5 log CFU/g population loss over the entire year tested. Population changes were detected at 25°C, with larger changes occurring at the higher humidity used. There was a significant difference ($P < 0.0001$) based on the variety of nut, with pine nuts showing approximately a 2 log CFU/g decline in *Salmonella* populations at 25°C and 57% RH versus 3.5 to 4-log decline under the same conditions for hazel nuts and pecans.

Significance: Differences in *Salmonella* survival on the different tree nut varieties may result in differences in the risk of illness associated with the consumption of these different nut varieties.

P2-113 Growth of Foodborne Pathogens on Inoculated Pistachios during Postharvest Handling

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Introduction: During harvest, pistachios are hulled and separated into streams based, in part, on nut density (sinker) and adhering hull (floater). Factors contributing to the observed higher prevalence of *Salmonella* in floater pistachios are unclear.

Purpose: To examine the behavior of *Salmonella, Escherichia coli* O157:H7, and *Listeria monocytogenes* on pistachios during simulated postharvest conditions.

Methods: In-hull and floater and sinker pistachios were collected from commercial processors. Samples were inoculated at 3 log CFU/g with cocktails of *Salmonella*, and in some cases *E. coli* O157:H7 or *L. monocytogenes* and incubated for up to 30 h under commercially-relevant conditions (37°C and 90% RH). Populations were measured by plating onto tryptic soy and selective agars.

Results: A 4- or 5-h lag and maximum populations of 7.2 (12 h) or 6.6 (8 h) log CFU/g were observed for *Salmonella* on early or late harvest in-hull pistachios, respectively. For hulled floaters and sinkers, no significant ($P < 0.05$) growth was observed in the first 3 h after inoculation. Thereafter, both the rate of growth and maximum populations differed between late and early harvest and among samples collected from different processors. Growth rates of *Salmonella* (0.38 and 0.24 log CFU/g/h, respectively) were significantly ($P < 0.05$) greater on floater compared to corresponding sinker pistachios and on floater pistachios with hull adhering to >25% of the shell surface. Maximum *Salmonella* populations were 2 log CFU/g greater on floaters than on sinkers. The growth of *E. coli* O157:H7 and *Salmonella* on hulled pistachios were similar but a longer lag (approx. 12 h) and significantly ($P < 0.05$) lower maximum population (4.3 log CFU/g) was observed for *L. monocytogenes.*

Significance: The growth of foodborne pathogens on pistachios during harvest can be managed by reducing the time between harvesting and hulling and from hulling to drying.

P2-114 A Mathematical Modeling Approach to the Evaluation of Three Sampling Plans for the Detection of Pathogenic Bacteria on Preharvest Leafy Greens

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Introduction: Recent outbreaks of foodborne disease associated with leafy greens has led to increased preharvest testing for pathogens and indicator organisms. However, the scientific and statistical rationale and performance attributes for the sampling plans employed have largely not be evaluated.

Purpose: The goal of this study was to develop a simple tool for evaluating the performance characteristics of three commonly used preharvest sampling plans: random, stratified-random, and z-pattern sampling.

Methods: Mathematical derivations and computer simulations by Matlab were performed to compare the relative effectiveness of random, stratified-random, and z-pattern sampling. This initial evaluation included consideration of both the number of contamination sites in the field and the number of samples analyzed.

Results: The detection probability with increasing number of contamination sites and increasing number of sample. When a large number of simulations were performed, the results agree well with the theoretical derivations, i.e., there was no difference on the mean detection probability for the three sampling plans. The detection probability decreased rapidly as a function of total number of subplots tested, in roughly a linear manner on log-log plots. However, the inherent variability of the z-pattern sampling plan was substantially greater than the other sampling plans. This difference is most dramatic when the number of contamination sites is small, with the z-pattern sampling plan having a high frequency of no-detection responses. Thus, the z-pattern sampling plan inherent variability has an increased probability of type 1 and type 2 errors compared to either random or stratified-random sampling plans.

Significance: This study provides a simple mathematical approach for evaluating the effectiveness three commonly used preharvest sampling plans, and suggest that with low level contamination, random or stratified-random sampling plans would likely be more effective.

P2-115 Cost Modeling of Biocontrol *Pseudomonas chlororaphis* and *P. fluorescens* for Competitive Exclusion of *Salmonella enterica* on Tomatoes

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Introduction: Produce-related outbreaks caused by *Salmonella enterica* and other pathogens are major constraints to food safety. Biological control of foodborne pathogens may complement physical and chemical post-harvest intervention measures to enhance food safety of minimally processed produce.

Purpose: The purpose of this research was to develop cost model estimates for competitive exclusion microbes (CEM) of *Pseudomonas* strains (non-plant pathogenic and non-human pathogens) as biocontrol of *S. enterica* on tomatoes. Published research on process-based models for biocontrol of foodborne pathogens on produce at post-harvest is limited.

Methods: Cost-estimates of competitive exclusion process were based on material inputs, equipment and facilities for application of biocontrol microbes, and projected processing conditions of post-harvest packaging of tomatoes or vegetables. The microbiological data for inactivation of *S. enterica* was based on published data from biocontrol experiments. Cost estimates of small and large scale applications of biocontrol and processing of tomatoes were compared.

Results: Initial capital investment costs for small-scale (assumed to have processing capacity of 2,000 Kg of tomatoes/h, 16 h/day, 6 days/week and 3 month/year) was US$ 391,000. For a large-scale facility, operating at 100,000 Kg of tomatoes/h for operational period, cost estimates were US$ 2.1 million. Application of CEM for biocontrol of *S. enterica* on tomatoes was estimated at US$ 0.058-0.073 kg of tomatoes. This cost can exceed other technologies such as chlorine dioxide.

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Significance: Cost estimate for CEM was developed. CEM applied against *S. enterica* on tomatoes may be attributed to microbial competition for space /nutrients, antagonism and antimicrobial compounds. For high value produce, CEM may complement existing post-harvest technologies, increase food safety, reduce loses and extend shelf life of produce if efficacy and delivery systems can be optimized.

P2-116 Development of a Dynamic Model to Describe the Kinetic Behavior of *Escherichia coli* in Diced Radish Kimchi

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❖ ***Developing Scientist Competitor***

Introduction: Some serotypes of *Escherichia coli* are foodborne pathogens, and the pathogens have been isolated from various fresh produces, which are used as kimchi ingredients. Hence, it is necessary to predict the fate of *E. coli* during kimchi fermentation.

Purpose: The objective of this study was to develop a dynamic model to describe the kinetic behavior of *E. coli* in diced white radish kimchi at changing temperatures.

Methods: Prepared diced white radish kimchi samples were inoculated with a five-strain mixture of *E. coli* at 4 log CFU/g. The samples were stored at 4°C (16 days), 15°C (4 days), and 25°C (2 days). Total lactic acid bacterial and *E. coli* cell counts were enumerated on de Man, Rogosa and Sharpe agar and 3M Petrifilm, respectively. The data of *E. coli* survival were fitted to the Baranyi model to calculate death rate (log CFU/g/h) and shoulder period (h). The kinetic parameters were further analyzed by a polynomial equation as a secondary model, and dynamic model was subsequently developed with changing temperatures. The model performance was validated with observed data, and root mean square error (*RMSE*) was calculated.

Results: Lactic acid bacteria in diced radish kimchi grew at all storage temperatures. However, *E. coli* growth was not observed at 4-25°C, and their cell counts were decreased during fermentation. As storage temperature increased, shoulder period decreased and the death rate increased ($P<0.05$). The developed kinetic models showed good performance with 0.480 of *RMSE*, and the prediction of the dynamic model showed also appropriate performance.

Significance: These results indicate that the developed dynamic model should be useful in describing the kinetic behavior of *E. coli* in diced white radish kimchi fermentation.

P2-117 Effect of Sanitizers on the Survival of Antibiotic-resistant Bacteria Applied to Raw Carrots through Contaminated Compost

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Introduction: It has been established that raw vegetables are associated with outbreaks of human illness, however the potential for fresh vegetables to serve as a vehicle for antibiotic-resistant bacteria is poorly understood. Antibiotics and antibiotic-resistant bacteria have been shown to persist in manure of dosed animals, and in compost made from this manure, where they may be transferred to produce.

Purpose: To determine the survival of antibiotic-resistant bacteria on raw, peeled, carrots after washing with commonly used sanitizers.

Methods: Multi-drug resistant *E. coli* O157:H7 and *Pseudomonas aeruginosa* were spiked into a compost slurry in order to inoculate carrot surfaces with a background microbial flora containing defined antibiotic-resistant bacteria relevant to produce. Carrots (n=3, 25 g) were air-dried and stored at 4**°**C until washing with sodium hypochlorite (50 ppm free chlorine) or peroxyacetic acid (50 ppm free peracetic acid), according to manufacturer's directions. Antibiotic-resistant bacteria were enumerated by serial dilution and plating onto antibiotic-supplemented R2A, R2A, Eosin Methylene Blue or *Pseudomonas* Isolation Agar.

Results: Reduction of *E. coli* O157:H7 and *P. aeruginosa* on carrots when washed with sodium hypochlorite were 3.27 log CFU/g and 1.65 log CFU/g, respectively, a significant difference in effectiveness between microbes ($P<0.05$). Washing with peroxyacetic acid reduced the numbers of *E. coli* O157:H7 and *P. aeruginosa* on carrots by 2.67 log CFU/g and 2.53 log CFU/g, respectively. Heterotrophic bacteria resistant to cefotaxime (10 µg/ml) reduced from 5.96 log CFU/g on cefotaxime-supplemented R2A plates (10 µg/ml) to 2.5 log CFU/g after washing with either sodium hypochlorite or peroxyacetic acid. A 4-log reduction in heterotrophic bacteria resistant to clindamycin (25 µg/ml) also occurred after sanitizer washes.

Significance: Results suggest that bacterial populations are altered by post-harvest washes and may select for antibiotic-resistant bacteria, indicating that vegetables may be an under-recognized source of antibiotic-resistant bacteria in the human diet.

P2-118 The Prevalence of Antibiotic-resistant Bacteria in Fresh Produce Purchased from Farmers Markets and Grocery Outlets

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Introduction: Antibiotic-resistant bacteria present a growing public health challenge. Although a number of sources of antibiotic-resistant bacteria have been identified, very little is known about the presence of antibiotic-resistant bacteria on fresh fruits and vegetables.

Purpose: The purpose of this study was to assess the presence of antibiotic-resistant bacteria on fresh produce acquired at farmers markets and a grocery store in Virginia.

Methods: Nine different produce commodities (180 total samples) from five different farmers' markets and a retail store were separately homogenized and enriched in tryptic soy broth. Homogenates were spread plated on selective media for *Enterococci* and enteric bacteria. Typical colonies were tested for susceptibility to tetracycline, sulfamethoxazole, ceftazidime, trimethoprim, ampicillin, vancomycin, and gentamicin using the Kirby Bauer disk diffusion assay. The zone of inhibition around each antibiotic was measured. Susceptibility limits were determined using CLSI Document M100-S21 (M2).

Results: Produce isolated enteric bacteria were resistant to tetracycline (68%), sulfamethoxazole (71%), ceftazidime (69%), and trimethoprim (94%). The *Enterococci* isolated were resistant to vancomycin (49%), ampicillin (32%), and gentamicin (27%). Produce samples traditionally grown touching soil (e.g., green onion) had a significantly greater number of enteric bacteria resistant to ceftazidime ($P = 0.009$) and trimethoprim ($P = 0.03$) when compared to produce that does not touch the soil (e.g., tomato). Proximity to soil did not influence enterococci resistance. Generally there were no

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differences in bacterial resistance profiles between products purchased from farmers' markets versus the grocery; however, more isolates recovered from samples purchased at farmers' markets were susceptible to ampicillin when compared to retail samples ($P < 0.05$).

Significance: Antibiotic resistant profiles from bacteria recovered from fresh produce may provide important information. Identifying the prevalence of antibiotic resistant bacteria in fresh produce may raise awareness of potential environmental contamination routes.

P2-119 Ampicillin Selection of *Listeria monocytogenes* Mutants Unable to Replicate on Rind of Fresh Cantaloupe

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Introduction: Several outbreaks of listeriosis have implicated fresh produce. However, *Listeria monocytogenes* (LM) genes required for growth on produce remains poorly understood. Such genes can be identified via localization of transposon insertion in transposon mutants unable to grow on produce. We hypothesized that such mutants will be selected from mutant libraries since ampicillin (β-lactam antibiotic) kills only growing cells.

Purpose: To assess the capacity of ampicillin selection to identify LM transposon mutants unable to grow on cantaloupe rind.

Methods: L1E4 is a cold-sensitive, erythromycin-resistant mutant of the erythromycin-susceptible strain 2858, implicated in the 2011 cantaloupe outbreak of listeriosis. Rind fragments of fresh cantaloupe were spot-inoculated with L1E4, 2858 and a 1:1 mixture of the two strains. Inoculated fragments were placed in Petri dishes with 10 ml sterile H_2O with or without ampicillin (100 µg/ml) so that inoculated rind surfaces remained immersed in the solution and incubated for 14 d at 4°C, a temperature that permits growth of 2858 but not L1E4. LM populations were enumerated on selective media (MOX) at 0, 7 and 14 d. Populations from fragments inoculated with mixed cultures were screened for erythromycin resistance to determine L1E4: 2858 ratios.

Results: Populations of the cold-sensitive transposon mutant L1E4 remained unchanged on the rind at 4°C with or without ampicillin. However, the proportion of L1E4 from fragments inoculated with mixed cultures increased from 47% at day 0 to 58 and 73% at 7 and 14 d, respectively ($P< 0.001$). Findings suggested that growing 2858 cells on cantaloupe were killed with ampicillin while non-replicating L1E4 cells were uninhibited.

Significance: Findings support value of the ampicillin selection protocol in screening mixed transposon mutant libraries to isolate non-replicating mutants on produce. Identification of LM genes essential for growth on produce will contribute to science-based strategies to enhance produce safety.

P2-120 Assessing the Potential for Antibiotic-resistant Bacteria and Resistance Genes to Carry Over from Soil Amendments to Vegetable Surfaces: A Greenhouse Study

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Introduction: Cattle are often treated with antibiotics, with their manure commonly used as a soil amendment. Little is known about the potential for antibiotic resistant bacteria (ARB) present in their manure to persist in soil and influence the microbial composition of vegetables. Composting may reduce risk of transferring manure-borne ARB to vegetable surfaces.

Purpose: A greenhouse study was carried out to determine the effect of application of composted manure, originating from cattle with or without antibiotic administration, on ARB found on the surface of radish taproots.

Methods: Manure was collected from treated and non-treated beef cattle (chlortetracycline, sulfamethazine, and tylosin) or dairy cattle (cephapirin and pirlimycin). Composting procedures followed the FDA Food Safety Modernization Act (FSMA) guidelines. Manures and composts were applied to locally-sourced soil. Radishes ($n = 3$) were grown to maturity, and the surfaces of the taproots were plated onto R2A containing clindamycin, ceftazidime, erythromycin, tetracycline, sulfamethoxazole, vancomycin, or no antibiotics to enumerate heterotrophic ARB.

Results: Radishes grown without soil amendment had fewer ceftazidime-resistant bacteria than those grown in soil amendments originating from beef ($P < 0.02$) or dairy ($P < 0.04$) cattle. Radishes grown in either compost also had more ceftazidime-resistant bacteria than those grown without soil amendment ($P < 0.006$). Radishes grown in beef compost and beef manure had greater tetracycline-resistant bacteria than those grown in dairy compost and dairy manure ($P < 0.01$). Tetracycline-resistant bacteria on radishes grown in manure were fewer than on radishes grown in compost ($P < 0.02$) and no soil amendment ($P < 0.05$).

Significance: This study can determine whether composted manure can be a source of ARB on fresh produce, providing insight as to how agricultural practices could spread ARB from the environment to humans. Strategies using practices, like those outlined in the FSMA produce rule, could reduce consumer exposure to ARB.

P2-121 Whole Genome Analysis of a Shiga Toxin-negative *Escherichia coli* O157:H7 Strain C1-057 Isolated from Feedlot Cattle

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Introduction: Shiga toxin production of Shiga toxin is one of the classical virulence factors of *Escherichia coli* O157:H7. A variant *E. coli* O157:H7 strain, C1-057, was isolated from feedlot cattle. Multiplex PCR confirmed that this strain carried neither *stx1* nor *stx2* genes (*stx*-negative).

Purpose: The whole genome of the Shiga toxin-negative *E. coli* O157:H7 strain was sequenced to understand the specific properties that are related to the colonization and adaptation of *E. coli* O157:H7 strains to feedlot cattle.

Methods: The C1-057 strain was cultured in trypticase soy broth overnight at 37°C and the genomic DNA was extracted. A Pacific Biosciences RSII system was used to obtain the complete genome sequences. A 3-20 kb library of the strain was prepared and sequenced, achieving average genome coverage of >100×. The 3-20 kb continuous-long-read (CLR) data were de novo assembled using the PacBio hierarchical genome assembly process 2 (HGAP2)/Quiver software package. Prophages and putative phage-like elements in the newly sequenced C1-057 strain were analyzed using the prophage-predicting PHAST Web server and were further compared with *E. coli* O157:H7 Sakai and *E. coli* K-12.

Results: When fully assembled, the complete genome consisted of the chromosome of 4,783,867 bp and two plasmids of 96,420 bp and 13,853 bp. These sequences were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP). These sequences have been deposited at GenBank under the accession LAZO00000000. The C1-057 strain shared eight similar predicted prophages with *E. coli* O157:H7 Sakai and shared three similar predicted prophages with *E. coli* K-12.

Significance: The distribution of similar types of prophages among *E. coli* genomes may underpin a possible path that *E. coli* genomes evolve from non-pathogenic to pathogenic strains. Genomic characterization of this variant Shiga toxin-negative strain may help to develop interventions to prevent the colonization of *E. coli* O157:H7 in feedlot cattle.

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P2-122 Effect of Calcium Hydroxide Application to Cattle Feedlot Pens on *Escherichia coli* O157:H7 and Total *E. coli* in Pen Surface Manure

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Introduction: Cattle and beef products are sources of the pathogen *Escherichia coli* O157:H7. Lime products have a long history of use in cattle production as disinfectants for sick pens, calving pens, and muddy areas, to control the spread of diseases. Lime may also be useful as a preharvest treatment for reducing cattle exposure and colonization by *E. coli* O157:H7, thereby reducing risk for beef contamination and human illness.

Purpose: The objective was to determine if hydrated lime ($Ca(OH)_2$) can reduce the levels and persistence of *E. coli* O157:H7 and total *E. coli* on feedlot pen surfaces.

Methods: Cattle were removed from 5 feedlot pens. Four 3 × 6-m plots in each pen were randomly assigned such that one plot of $Ca(OH)_2$ treatment rates of 0, 2.5, 5, and 10% (w/w) was present in each pen. $Ca(OH)_2$ was incorporated into feedlot surface manure (FSM) using rakes. Five FSM samples were collected from each plot at 0, 1, 7, 14, 21, and 28 d and analyzed separately ($n = 25$). Total *E. coli* concentrations were determined by direct plating, and *E. coli* O157:H7 presence was determined by enrichment, immunomagnetic separation, and plating.

Results: *E. coli* O157:H7 prevalence in FSM ranged from 0 to 8% from day 0 to day 7, with no difference by treatment ($P > 0.05$). *E. coli* O157:H7 was not detected in any FSM treatment on or after day 14. Concentrations of total *E. coli* in FSM were 2.07 and 2.65 log CFU/g lower ($P < 0.05$) immediately after application of 5 and 10% $Ca(OH)_2$, compared to 0% $Ca(OH)_2$. However, there was no difference in total *E. coli* concentrations in any treatment on day 7 or after ($P > 0.05$).

Significance: Further work is needed to identify $Ca(OH)_2$ levels or alternative lime products to reduce *E. coli* O157:H7 in cattle and production environments.

P2-123 Phenotypic Characterization of Antimicrobial Resistance in *Salmonella enterica* Isolates Associated with Cattle at Harvest in Mexico

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Introduction: In recent years, the emergence of antimicrobial resistance in *Salmonella* has become a major public health concern worldwide. *Salmonella* is a common food contaminant that can develop resistance to antimicrobials used to treat human or animal illnesses. Consequently, there is an increasing concern regarding the use of antimicrobials in food animals such as beef cattle, since this could be a potential factor in the dissemination of antimicrobial-resistant *Salmonella*.

Purpose: The objective was to determine the antimicrobial susceptibility profiles among *Salmonella enterica* isolates from cattle presented at harvest in Mexico.

Methods: A total of 351 *Salmonella* isolates from fecal samples (n=31), beef hides (n=105), and beef carcasses (n=215), were included in the study to test for antimicrobial susceptibility. Isolates were isolated from samples collected in three abattoirs in Mexico. Susceptibility profiles were determined using the Sensititre automated antimicrobial susceptibility system with the Gram Negative NARMS plate format CMV3AGNF. The MIC breakpoints for each of the 14 antimicrobials tested were interpreted using the CLSI standards for microdilution broth methods, when available, and with the NARMS breakpoints.

Results: Overall, a total of 205 (58.4%) *Salmonella enterica* isolates tested exhibited resistance to at least one or more antimicrobial. Furthermore, all 351 isolates exhibited 20 different resistance phenotypes. Resistance to tetracycline (40.2% of the isolates were resistant), and resistance to nalidixic acid (21.1%) were observed most often. Additionally, the most common multidrug-resistant phenotypes present in the isolates shared resistance to chloramphenicol, streptomycin, tetracycline, and trimethopin/sulfamethoxazole (11.3%), resistance to ampicillin, tetracycline, and trimethopin/sulfamethoxazole (3.4%), and resistance to ampicillin, streptomycin, and tetracycline (2.5%).

Significance: Understanding the diversity of antibiotic-resistant *Salmonella* isolated from food animals can promote control measures in livestock production practices globally. This can subsequently aid in the prevention of the spread of antimicrobial resistance pathogens such as *Salmonella* to human environments.

P2-124 Prevalence of Foodborne Pathogens in Livestock Raised on Small-scale Farms in California

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❖ ***Developing Scientist Competitor***

Introduction: The increasing popularity of small-scale farms reflects growing consumer interest in local food production and sustainability, including humanely produced meat and eggs. However, livestock harbor foodborne pathogens that can cause major illness.

Purpose: To identify on-farm food safety practices that are specific to the unique conditions and needs of small-scale and/or bio-diversified farms (i.e., operations that integrate livestock and fresh produce). The objective is to assess risk factors and the prevalence of certain foodborne pathogens in livestock raised on small-scale diversified farms.

Methods: Twenty-one farms in California were enrolled in this cross-sectional study conducted in 2015. Seventeen (81.0%) were bio-diverse farms and 4 raised only livestock. Eleven raised more than one species (52.4%) and 18 (85.7%) keep poultry. Individual livestock feces and composite poultry samples were collected from the ground and were cultured for *Campylobacter*, Shiga toxin-producing *Escherichia coli* (STEC), and/or *Salmonella;* selected isolates were serotyped.

Results: Foodborne pathogens were found on 71.4% (15/21) farms at least once during the study. Out of 626 random samples collected, 301 (48.1%) were poultry and the remaining 325 were from non-poultry livestock. The overall *Campylobacter* prevalence was 6.9% (43/626) including isolations from poultry, swine, cattle, goats, and sheep. STEC O157:H7 was found in 1.9% (6/325) samples overall and all isolates were from beef and dairy cattle at one ranch. Prevalence of non-O157 STEC was 7.7% (25/325) including detections in cattle, goats and sheep. Overall prevalence of *Salmonella* was 0.8% (5/626), and all isolates were from chickens at two farms. STEC serogroups included O103, O111, O136, O182, O26 and O43. *Salmonella* serovars included *Salmonella* Agona, *Salmonella* Othmarschen and *Salmonella* Braenderup.

Significance: This study highlights the need to assess potential food safety risks associated with small-scale, pasture-based, diversified farms. Findings will provide scale-appropriate food safety metrics and recommendations for risk reduction to farms integrating crops and livestock.

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P2-125 Prevalence of Microbial Threats in Dairy Production According to the Cattle Feeding System

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Introduction: Interventions aimed upstream of the food chain can help to reduce at the source microbial contaminants associated with foodborne diseases such as non-typhoidal *Salmonella*, enteropathogenic *Escherichia coli* and *Listeria monocytogenes*. These microorganisms are recognized as the main causative agents of gastroenteritis in Canada. In fact, farm characteristics and management practices can affect the abundance of bacterial pathogens in a given herd. The component-fed rations (CFR) feeding system could represent a potential risk factor for an increased prevalence of foodborne pathogens, as it is frequently distributed manually by the producer, compared to a total mixed rations (TMR) feeding system, which is often distributed automatically.

Purpose: To determine whether the use of CFR systems, compared to TMR systems, can influence the prevalence of three foodborne microorganisms in animals from dairy herds.

Methods: Ten similar dairy cattle farms using a CFR system (n=5) or a TMR system (n=5) were each visited four times over a one-year period. Rectal samples (50 g) were collected from 15 lactating cows per farm per visit for a total of 600 samples. Using the appropriate selective media, *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes* were isolated from the feces collected. Confirmatory bacterial identification was performed by PCR on isolated colonies.

Results: The prevalence of *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 in CFR-fed animals (n=300) were 5.7%, 7.7%, and 1.7%, respectively, compared to 1.3%, 8.3%, and 0% in TMR-fed animals (n=300). The difference was statistically significant for *Salmonella* (*P*=0.004) and for *E. coli* (*P*= 0.025). The peak in detection occurred during spring for all pathogens.

Significance: In this study, the CFR feeding system is associated with a higher prevalence of certain microorganisms. Characterizing production practices is still necessary in order to implement effective control strategies for bacteria that can affect food safety.

P2-126 Assessing the Role of Farm Hygiene as Predictor of Milk Contamination by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in Dairy Farms

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Introduction: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is associated with Johne's disease in dairy cows, and is suspected to be linked to Crohn's disease in humans. MAP can be secreted by cows with milk (internal route), or contaminate milk via contact with feces or farm equipment (external route). Humans can be exposed to MAP via milk consumption, since MAP is not fully inactivated by pasteurization. While hygiene measures aim to limit milk contamination, to-date the relative importance of different contamination routes is poorly understood.

Purpose: This study aims to: 1) Survey hygiene practices in Northeast U.S. dairy farms, 2) Identify farm practices and hygiene interventions that impact MAP contamination in bulk milk, and 3) Develop a model of MAP transmission in dairy farms, with focus on herd and environmental hygiene practices.

Methods: Cross-sectional data on MAP and corresponding antibodies in bulk milk, as well as herd and farm hygiene metrics were collected for 292 dairy farms in the Northeast U.S. Classification and regression tree (CART) analyses were used to identify major risk predictors.

Results: Milk quality metrics, in particular somatic cell counts and plate counts in milk, cow-side somatic cell counts, and levels of *E. coli* and *Streptococcus* in milk were the main predictors of MAP antibody levels in milk, as assessed by ELISA assay (CART importance rank = 4-7). Age of cow housing facilities, breed, detection of *Mycoplasma* in milk, and high use of pasture were also of intermediate importance (rank = 3). Only in 2.1% of farms was MAP detected in milk by PCR, making this variable unsuitable for CART analysis.

Significance: Findings suggest that both animal health and farm hygiene can play an important role in MAP contamination in milk. Further quantifying these variables could inform risk assessment models and improve milk quality.

P2-127 Presence of *Salmonella*, *Escherichia coli* O157 and *Campylobacter* in Small Ruminants

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Introduction: Meat derived from goats and lambs an often underappreciated protein source in the U.S., but especially important in developing countries challenged with food insecurity. Extensive studies have examined prevalence of *Salmonella, Campylobacter* and *Escherichia coli* O157 in beef, pork and poultry species; however, less research is available about pathogen presence in small-ruminants. Understanding the presence of these pathogens in small-ruminants can influence processing method improvements for these species to minimize food safety risks.

Purpose: This study had three main objectives: 1) Determine pathogen presence in small-ruminants on hide and fecal samples collected from U.S. and international sources; 2) *Salmonella* presence in small-ruminant carcass surface and retail samples from the U.S. and international sources; and 3) *Salmonella* presence found in lymph nodes from sheep and goats.

Methods: Samples were collected at abattoirs and farms located in California, New Mexico, Texas, the Bahamas and Mexico over a 14-month period. Samples were processed using a combination of traditional culturing methods and real-time PCR. All presumptive positive samples were confirmed by isolation and biochemical analysis.

Results: *Salmonella* was detected in 17.11% of hide samples (*N* = 339), 13.91% of fecal samples (*n* = 532), 16.82% of retail samples (*n* = 106), and 1.94% - 9.62% of lymph nodes (*n* = 668) and up to 5.20% on carcass surfaces (*n* = 878). *E.* coli O157 was present in 1.50% of hide samples (*n* = 266), and 15.30% of fecal samples (*n* = 477). *Campylobacter* was found in 80.68% of fecal samples (*n* = 176).

Significance: The results indicate the presence of potential pathogens from small-ruminant sources. Further study of the trends of these pathogens through season, geographical location and management conditions, as well as control measures, are important to better understand the risks associated with these pathogens present in small-ruminants.

P2-128 Adhesion of Avian Pathogens to Enterocyte Cell Line after Adaptation to Gastrointestinal Environment

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Introduction: The expression of membrane-associated adhesins which allow specific cell-cell interactions that can lead to adhesion and colonization is tightly controlled. During gastrointestinal (GI) transit, pathogens encounter environmental stresses (pH, bile salts, nutrient limitation, microaerobie, etc.) that affect expression.

Purpose: To evaluate the effect of GI transit on the bacterial adhesion to enterocyte cell line (Caco-2).

Methods: Caco-2 cells were cultured in DMEM, seeded onto Transwell membrane and incubated to allow the formation of differentiated monolayer. Pathogens were adapted to in vitro GI environment: a 1-h in vitro gastric digestion process followed by an intestinal digestion of 1 h. Adapted bacteria (8 log CFU/ml) were transferred to cell monolayer (MOI 100:1) and incubated for 1 h. Caco-2 were washed with PBS to remove non-adherent bacteria, and treated with Triton-X100 to dissociate adherent bacteria, which were then counted. The % adhesion (adherent bacteria divided by total bacteria × 100) for adapted and intact bacteria was compared.

Results: Three avian clinical strains *Escherichia coli* Tk301:k1 and O78:H80 and *Salmonella enterica* var. Typhimurium DT104 were tested. The overall % adhesion (1.06 ± 0.82%) varied greatly among strains and test days (range 0.33 to 3.1%). For all strains, bacterial adhesion with and without in vitro GI transit treatment did not differ significantly.

Significance: Although bacterial adaptation to GI environment can induce the expression of adhesins and other bacterial surface components, this did not improve early adhesion of pathogens. This suggests that adhesins and other specific surface proteins do not play significant role during the initial stage of adhesion. Initial contact between bacteria and cells appears to be mainly non-specific and dependent on the hydrophobicity and surface charge of bacterial cells.

P2-129 Use of Dean Flow Ultraviolet (UV) Reactors for the Cold Pasteurization of Tender Coconut Water

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Introduction: The natural water inside the green coconuts is regarded as a healthy drink due to the elements of nutritional and therapeutic value. Since there is chance of contamination of tender coconut water (TCW) during extraction from its hard shell with psychrophilic microbes if they are stored at 4°C, thermal pasteurization is currently practiced. However, the thermal treatment of TCW causes rise in off flavors and loss of the vital nutrients. To solve this problem, a non-thermal pasteurization technology is desirable.

Purpose: The goal of this research was to assess the antimicrobial effectiveness of Ultraviolet light (UVc) as cold pasteurization of TCW and evaluation of physico-chemical and sensory quality of the treated TCW in comparison to the fresh TCW.

Methods: A dean flow ultraviolet reactor with wavelength of 200-280 nm at the residence time of 14.2 seconds was used. The independent variables were 3 Reynold numbers (Re) and 2 diameters of transparent PFA tubes (1/8" and 1/16"). The antimicrobial effect was evaluated with *E. coli* W1485 as a model organism for cold pasteurization. Physico-chemical and sensory quality of TCW were tested and analyzed throughout the storage period of 4 weeks.

Results: UVc treatment was significantly effective to inactivate *E.coli* W1485 ($P < 0.05$) in both the tube sizes. Also, the diameter of tubes and Reynold numbers significantly affected CFUs of *E.coli* in treated TCW as lesser diameter (1/16") tube was more effective (3.1, 4.2 and 5.3 log reduction) than 1/8" tube (1.9, 2.4 and 3.09 log CFU reduction) and higher the Re values better the inactivation observed for 47, 95 and 142 Reynold numbers. The physico-chemical and sensorial changes of cold pasteurized TCW was not significantly different compared to the fresh TCW.

Significance: Dean flow UVc reactor offers a potential cold pasteurization technology for tender coconut water.

P2-130 Thermal Inactivation of *Listeria monocytogenes* in Bovine and Non-bovine Milk Pasteurization

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Introduction: Non-bovine milks are becoming popular for fluid milk consumption and artisanal cheese production. *Listeria monocytogenes* (LM) is one of the most common pathogens in raw milk and has been found in 50% of commingled raw bovine milk silos in the US. Bovine milk pasteurization conditions should be adequate for proper treatment of non-bovine milks, however little research has been completed to define the differences in thermal resistance of LM in non-bovine milks.

Purpose: To compare the thermal inactivation of *Listeria monocytogenes* in bovine, goat, camel, and water buffalo milks using standard HTST pasteurization conditions.

Methods: A preliminary screening of 25 LM strains was conducted by heating each in 6 ml TSB+YE at 60°C in submerged glass vials for up to 30 min with quantitation on TSA+YE. The most resistant strain was used for inactivation studies in which 5 ml milk in glass vials was pre-heated at 71.7°C for 3 min prior to inoculation with 20 µl of ~10^{10} CFU/ml LM via syringe. After heat treatment, the vials were moved to an ice slurry for 30 s followed by dilution and plating on TSA+YE. Four trials per milk, with duplicate vials for each treatment time (0, 2, 4, 6, 8, 10, 12, 14, and 16 s) were quantitated and the time to reduce LM by 1 log (*D*-value) was determined.

Results: Average *D*-values were 5.92±0.56 s, 5.36±1.10 s, 4.58±0.82 s, and 3.67±0.20 s for LM in water buffalo, goat, bovine, and camel milks, respectively. *D*-values increased with increasing levels of fat, protein and solids. Only buffalo milk was significantly different ($P<0.05$) than bovine milk, and contained the highest percentage of fat, protein and solids.

Significance: LM in goat and camel milks was inactivated similarly to LM in bovine milk while buffalo milk may require a longer treatment time or a higher temperature to result in the same reductions.

P2-131 Thermal Inactivation of *Coxiella burnetii* and *Micrococcus luteus* in Bovine and Non-bovine Milk Pasteurization

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Introduction: Non-bovine milks are becoming popular for human consumption and cheese processing. Understanding how pathogens are inactivated in these milks using standard HTST pasteurization conditions is significant for food safety. A liquid culture-based MPN-PCR assay has been developed to quantitate the pasteurization reference organism, *Coxiella burnetii*, in milk, however a more easily quantitated surrogate organism would be advantageous.

Purpose: To quantitate *Coxiella burnetii* and *Micrococcus luteus* (*M*L) thermal inactivation at 71.7°C in bovine, camel and goat milks and evaluate *ML* as a potential surrogate.

Methods: Milk (6 ml) was pre-heated in a water bath to 71.7°C in vials for 2.5 min. prior to co-inoculation of *CB* and *ML* at a final concentration ~ 6.5 log ge and CFU/ml using a syringe. Inoculated vials were heat-treated at 71.7°C up to 16 s, cooled in an ice slurry and serially diluted. Viable *ML*

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was quantitated by plating on BHIA. For *CB* enumeration, 1 ml each dilution was inoculated into 9 ml Acidified Citrate Cysteine Medium-2 (ACCM-2) in triplicate T-25 flasks to produce a 3-tube Most Probable Number (MPN) assay and incubated for 14 days at 37ºC under modified atmosphere. The number of flasks showing growth of > 0.5 log ge/ml by qPCR were used to calculate the *CB*MPN/ml milk. Two trials with two vials per treatment time were completed for each milk.

Results: Average *D*-values were 1.99 ± 0.21 s, 2.06 ± 0.71 s and 1.43 ± 0.30 s for *CB*, and 5.47 ± 0.94 s, 5.34 ± 1.54 s, and 3.48 ± 0.83 s for *ML* in bovine, goat, and camel milks, respectively. The *D*-values of the non-bovine milks were not significantly different ($P<0.05$) than bovine milk for both strains. *ML* and *CB* were inactivated to a greater degree in goat milk than camel milk.

Significance: *CB* inactivation in non-bovine milks was similar to that of bovine milk. Although conservative, *ML* may act as an appropriate surrogate for estimating *CB* inactivation in milks.

P2-132 Bioluminescence as Alternative Rapid Method for the Detection of Heat-resistant Sporulated Microorganisms from UHT Milk: A Case Study

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Introduction: Rapid technologies, such as bioluminescence 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 3M Microbial Luminescence System (MLS II) is a rapid alternative. A UHT-milk producer in Cordoba, Argentina detected through the 3M MLS II, the presence of microbial contamination after 72 hours of incubation, however enrichment and analysis of samples after seven days utilizing pH did not indicate the presence of microbial contamination.

Purpose: To investigate the root cause of discrepant results between bioluminescence, growth on agar and pH for the evaluation of microbial contamination in UHT milk.

Methods: Fifteen samples of UHT-milk were analyzed following incubation at 37°C/72 h, using the 3M MLS II, streaking on nutrient agar and pH. Three samples of UHT-milk from lots presumptively contaminated, according to bioluminescence analysis, were collected and further analyzed to investigate the root cause of the discrepant results between bioluminescence, and traditional methods. Samples were transferred to a glass bottle and incubated at 55°C/48 h. After incubation 300 ml of sample were plated on nutrient agar supplemented with Vitamin B12. A separate aliquot was heat-shocked at 80°C/10 min to activate germination of spore formers if present. Four isolated colonies were analyzed through MALDI to determine genus and species.

Results: Application of bioluminescence detected microbial contamination in UHT-milk samples that did not produce changes in physicochemical parameters such as pH or growth in traditional nutrient agar. Further treatment of enriched samples to promote spore germination and plating in agar supplemented with vitamin B12 allowed detection of *Geobacillus stearothermophilus* which is a heat-resistant spore former.

Significance: Bioluminescence technology provides rapid test results which are increasingly becoming important for evaluating commercial sterility in UHT-milk. These tests aim delivering faster time to results without sacrificing performance. Understanding performance and recovery across various methods provides valuable information that will help informed decision making towards the method of choice.

P2-133 Identification of Sporeforming Bacteria Isolated from a Condensed Milk Chain and Its Potential Entry Points

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Introduction: Sporeforming bacteria are heat resistant microorganisms capable of surviving and germinating in milk after pasteurization. They have been reported to produce lipolysis and proteolytic enzymes, even in low-temperature conditions, which affect the quality of dairy products. Therefore, the dairy industry is interested in extending the shelf life of its products by controlling sporeformers in early stages of the chain.

Purpose: The objective of this research was to determine the sources of potential "problematic" sporeforming bacteria present throughout a condensed milk processing chain, including sources at the farm level.

Methods: During two seasons, condensed milk samples from a commercial plant and environmental samples from three farms were collected and heat-treated (80°C/12 min) to recover only sporeformers. Samples were spread-plated using Standard Methods Agar (SMA) and incubated at 32°C. Samples were also stored at 7°C and 55°C, to determine which sporeformers had the ability to grow under refrigeration and high temperature conditions. Isolates were identified using molecular techniques (16S or *rpoB* sequencing).

Results: Molecular typing results showed that *Paenibacillus* spp. (*odorifer*, *cookii, graminis*) were responsible for the fluid milk spoilage due to its ability to grow at low temperatures. *Paenibacillus* was also found in condensed milk from the plant. Other *Bacillus* species found in condensed milk include: *B. claussi*, *B. subtilis*, *Lysinobacillus* sp., *B. safensis*, *B. licheniformis*, *B. sonorensis* and *Brevibacillus* sp. These last three species are capable of growing at thermophilic temperatures. Among the sources of "problematic" strains at the farm, it was found that milking equipment and cow teats contained a variety of psychrotrophic and thermophilic "problematic" strains, suggesting that interventions should be targeting these areas.

Significance: By detecting potential sources of these organisms at the farm level, farmers could design suitable interventions to decrease sporeformers counts in raw milk and ultimately improve the quality of condensed milk.

P2-134 Genetic Relatedness of Psychrotolerant *Bacillus cereus* Group Isolates from Dairy Sources

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❖ ***Developing Scientist Competitor***

Introduction: Members of the *Bacillus cereus* Group are genetically closely related and are indistinguishable by 16S rDNA sequence comparison, but may be characterized according to seven-gene multilocus sequence typing (MLST). Although species within the *B. cereus* Group are genetically closely related, phenotypic characteristics such as the ability to grow at cold temperatures vary by strain.

Purpose: The purpose of this study was to evaluate the genetic and phenotypic diversity of *B. cereus* Group strains isolated from all stages of the dairy value chain, including the dairy farm environment, and raw and pasteurized dairy commodities.

Methods: We selected a total of 48 *B. cereus* Group isolates from a frozen stock collection representing 41 unique *rpoB* allelic types and evaluated their ability to grow at 6°C. We performed MLST according to the *B. cereus* PubMLST scheme, and constructed a neighbor-joining tree based on (i) the *rpoB* gene sequences and (ii) the concatenated sequences of seven MLST housekeeping genes.

Results: The 48 strains represented 46 unique MLST sequence types. Of the 48 selected strains, 13 grew at 6°C. The isolates clustered into the same five clades based on both *rpoB* and MLST sequences. Of the 13 that grew at 6°C, 10 clustered within the same clade.

Significance: As some strains of the *B. cereus* Group produce toxins, the ability of some strains to grow at 6°C presents a potential food safety concern to the dairy industry, as they may cause milk spoilage and present risk for foodborne disease. This work suggests that *B. cereus* Group isolates that can grow at refrigeration temperatures largely represent a phylogenetically homogeneous sub-group.

P2-135 Genetic Diversity of *Bacillus sporothermodurans* Isolated in Africa and Europe

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Introduction: *Bacillus sporothermodurans* is a psychrotolerant, mesophilic, aerobic endospore-forming bacteria which may produce extreme heat-resistant spores. Their spores have been found to be more resistant than other heat resistant spores at temperatures above 130°C with D_{140} ranging from 3.4–7.9 s. The heat resistance in some strains is as a result of a heat resistant clone (HRS clone). The spores may contaminate milk and milk products and some processed soup products and their presence in foods may cause spoilage and contravenes good manufacturing practice. However, presence of the heat resistant clone may be location or matrix specific hence the importance to access the genetic relatedness of isolated strains isolated from different locations and food matrices.

Purpose: To confirm the presence of heat resistant clones of strains of *Bacillus sporothermodurans* and to determine their relatedness through GTG_5 fingerprinting.

Methods: Genomic DNA was extracted from bacterial cells grown on brain heart infusion agar and amplified through PCR using the BSPO and HRS primers, to confirm *B. sporothermodurans* and heat resistant clones, respectively. The GTG5 fingerprinting technique was performed using the GTGGT-GGTGGTGGTG primer. Phylogenetic analysis was preformed to compare relatedness of strains isolated in Africa and Europe.

Results: In the present study approximately 37.5% of *B. sporothermodurans* strains confirmed were positive for the HRS clone. The South African strains clustered away from each other but still showed between 85 and 93% similarity level. Some strains isolated from different matrices in Europe clustered together; generally exhibiting a 95 % similarity level.

Significance: There is gradual increase in the prevalence of *B. sporothermodurans* in especially milk products. Identifying genetic differences would help inform technologies on the inactivation of *B. sporothermodurans* and to subsequently reduce spoilage and economic losses in the dairy industry.

P2-136 Withdrawn

P2-137 Characterization of Toxin Gene Distribution and Toxin Production Provides Insight to the Potential Differentiation of Pathogenic and Non-pathogenic *Bacillus cereus* Group Strains

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❖ ***Developing Scientist Competitor***

Introduction: The *Bacillus cereus* Group consists of 9 highly genetically related species, some causing foodborne illness, and some being used as insecticidal agents or as probiotics for livestock. Current differentiation methods based on colony morphology and standard biochemical methods do not accurately differentiate between species, therefore methods allowing for better differentiation between potentially pathogenic and non-pathogenic strains within the *Bacillus cereus* Group is needed.

Purpose: The purpose of this study was to characterize the distribution of genes encoding toxins among *Bacillus cereus* strains, and to determine the expression of various virulence factors, including their expression at human body temperature.

Methods: A total of 66 bacterial isolates previously identified as belonging to the *B. cereus* Group using a 632 nt segment of the *rpoB* gene were characterized in this study. Briefly, the presence of 8 different toxin encoding genes were detected using PCR. The colony morphology of strains representing 43 unique *rpoB* allelic types was observed using Brain Heart Infusion and Bacara agar plates. The activity of phosphoinositide phospholipase C(PI-PLC) was characterized using *Bacillus cereus/Bacillus thuringiensis* chromogenic plating medium. Hemolysis activity was determined using the FDA BAM method. Growth at refrigerated temperatures was determined following 21 days incubation at 6-7°C. Finally, production of enterotoxins Nhe and Hbl was detected using Duopath® Cereus Enterotoxins (Merck) kits.

Results: Overall, the majority of isolates screened encoded all 8 toxin genes. Out of 43 strains, 40 were hemolytic, 39 showed PI-PLC enzymatic activity, and 13 were able to grow at cold temperatures. Morphology on both BHI and Bacara agar did not allow for differentiation among species within the group. Preliminary results indicate that the isolates tested produce toxins when strains are grown at 37°C.

Significance: These results provide detailed information for strains within the *B. cereus* Group, and may potentially contribute to improved differentiation of pathogenic and non-pathogenic strains within the *B. cereus* Group.

P2-138 Influence of Product Formulation on *D*250°F and *F*250°F Corrected Values of Clostridium sporogenes ATCC 7955

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Introduction: The *D*-value is the time (minutes) required to destroy 90% (1 log) of a given bacterial population at a constant temperature and is inversely related to the rate of destruction. In combination with other variables such as material heat penetration rate, the value is used to define thermal processing conditions necessary to render a specific product microbiologically safe. *D*-values are influenced by physiochemical factors such as pH, chemical composition and viscosity, so recalculation may be warranted when product formulations are changed significantly.

Purpose: This study evaluated the impact of product formula modification on the $D_{250°F}$ and $F_{250°F}$ corrected values of *Clostridium sporogenes* ATCC 7955 in order to support thermal process development.

Methods: A 2^4 factorial experimental design was implemented to determine the $D_{250°F}$ values for 16 formulations which changed protein mix (Milk Protein Concentrate (MPC) or Soy Protein Isolate (SPC)/MPC mix), or increased the protein, carbohydrate (Sucrose), and/or vegetable oil level, each by 10%, in a base formulation. The formulations were inoculated with *C. sporogenes* ATCC 7955 (final concentration of 2.0 x 10^6 spores/ml) and subjected

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to 250°F at different intervals using the Joslyn BIER unit. Survivors were enumerated on Modified PA 3679 agar, the $D_{250°F}$ and $F_{250°F}$ corrected value calculated. The statistical analysis evaluated both main effects and two-way interactions.

Results: Increased fat, protein, and soy protein content increased the $F_{250°F}$ corrected value by 0.5 minutes or more. However, under the conditions of the study, increased carbohydrate did not influence the $F_{250°F}$corrected values.

Significance: These results indicate that changing fat and protein content, and protein composition can significantly alter the $D_{250°F}$ and $F_{250°F}$ corrected values of *C. sporogenes* ATCC 7955 potentially influencing heat sensitivity.

P2-139 Evaluation of the Growth Potential of *Listeria monocytogenes* in Milkshakes Prepared with Contaminated Ice Cream Linked to a Listeriosis Outbreak and Stored at Room Temperature

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Introduction: A multistate listeriosis outbreak linked to contaminated ice cream products caused 4 illnesses, including 3 deaths of highly immunocompromised patients in a Kansas hospital. These patients consumed milkshakes prepared from the contaminated ice cream products. Previous enumeration work showed that *L. monocytogenes* was detected in 99.4% of the ice cream and 92.3% of the products were contaminated at a level below 20 MPN/g.

Purpose: We evaluated the growth potential of *L. monocytogenes* in milkshakes prepared with the contaminated ice cream products and stored at room temperature. This would help estimate the level of *L. monocytogenes* consumed by patients if these milkshakes were temperature abused in the hospital.

Methods: Three different flavors of milkshakes, vanilla, chocolate and strawberry, were prepared according to the receipt from the Kansas hospital and stored at room temperature (68°F and 73°F) for up to 15 hours. Milkshake samples were taken hourly and *L. monocytogenes* was enumerated by direct plating onto Rapid *L. mono* agars.

Results: The cell counts, used to construct growth curves of *L. monocytogenes* in these milkshake samples, demonstrated that the lag phase was 6.5 hours for milkshakes of all three flavors stored at both 68°F and 73°F. The generation time of *L. monocytogenes* in the exponential phase was 1 hour and 40 min in milkshakes stored at both 68 and 73°F. The results show that even if milkshakes were temperature abused at room temperature in the hospital, *L. monocytogenes* might not grow to much higher levels than the initial level.

Significance: The data provided valuable information towards the improved assessment of risk for listeriosis in highly susceptible populations and a better understanding of *L. monocytogenes* growth in ice cream left at room temperature.

P2-140 Evaluation of NBB-PCR: A Reliable, Fast and Universal Enrichment Broth for the PCR Detection of Beer Spoiling Microorganisms

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Introduction: The detection of microorganisms using rapid methods, like PCR or real-time PCR, have gained importance in a brewery's quality control. Although these methods permit fast and reliable detection of beer spoilers, the cultural enrichment of these samples is still a crucial step - especially for slow growing microorganisms. A suitable enrichment medium should induce fast microbial growth. We demonstrate a new culture medium, NBB-PCR Broth, with an optimized nutrient composition for enrichment of beer spoiling microorganisms (within 48 h), allowing an optimal detection with PCR or real-time PCR.

Purpose: The aim was to develop a fast, universal enrichment medium for all relevant beer spoilers that is compatible with all common PCR detection methods and sample types arising in a brewery, to ensure reliable results.

Methods: The new broth was tested with the strains *Lactobacillus brevis, Lactobacillus lindneri, Pediococcus damnosus* and *Pectinatus frisingensis* (100 cells/ml, n=3). Samples of filterable beer, cloudy beer, pitching yeast and cropped yeast (n=3 each) were spiked with 100 cells/sample of the respective microorganism. After incubation at 28°C, samples were analyzed with four real-time PCR systems, as well as a novel PCR based lateral flow detection system.

Results: The developed enrichment medium was compatible with all tested PCR or real-time PCR systems, with no false positive results or inhibition of PCR observed. After 48 h of enrichment, all tested microorganisms reached reliable detection levels or showed significant reduction in Ct-values. *L. brevis* and even slow growing *L. lindneri* were already detectable after 24 h (by one of the tested PCR systems).

Significance: NBB-PCR Broth is a reliable, ready-to-use enrichment medium that allows PCR or real-time PCR detection of beer spoilers (in 48 h), as demonstrated with four differing test strains. Its compatibility with all market-relevant PCR systems enables a dramatic decrease of the detection time and enables faster product release in the breweries.

P2-141 Farm Sources of *Listeria monocytogenes* and Impact on the Microbial Safety of Milk Destined for Artisan Cheese Production

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Introduction: FDA has requested scientific data and information to assist in identification and evaluation of intervention measures that have an effect on the presence of bacterial pathogens in raw milk cheeses.

Purpose: Determine overall presence of *Listeria* spp. on farms producing milk for artisan cheesemakers, with the primary goal of identification of management practices that accomplish mitigation of sources of contamination

Methods: Environmental sampling was conducted on five dairy farms that were either cheese producers or were supplying milk to artisan cheese producers. A double enrichment protocol was used to isolate *Listeria* spp. and multiplex PCR was used to identify each isolate. All *L. monocytogenes* isolates were ribotyped though the use of the DuPont RiboPrinter® System.

Results: Out of 266 samples, 33.0% tested positive for *Listeria* spp., and 16 samples tested positive for *L. monocytogenes* (6.0%). We compared *Listeria* incidence on four farms; two (Farms A and D) fed dry hay or fed cows on pasture, while two others (Farms B and C) fed silage to animals. In both Farms B and C, *L. monocytogenes* ribotypes DUP 1039C and 1061 were found in silage and areas of the farm environment, particularly in water sources. Presence of *Listeria* was not detected in bulk tank milk, but was detected in one milk filter.

Significance: Advising artisan cheesemakers making raw milk cheeses to eliminate silage feeding in favor of dry hay or pasture feeding is a strategy which shows promise to reduce presence of *Listeria* in milk. Testing milk filters for presence of *Listeria* may be more effective in identifying potential

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presence of *Listeria* in milk. *Listeria* is rarely present in raw milk used for artisan cheese production. When we have found presence, it is at levels below detection limits. Testing milk filters may increase detection sensitivity and provide confidence in negative results.

P2-142 FDA's Compliance Program Guideline Criteria for Non-toxigenic *Escherichia coli*: Impacts on Domestic and Imported Cheeses

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Introduction: The U.S. Food and Drug Administration's 2015 Domestic and Imported Cheese and Cheese Products Compliance Program Guidelines (CPG) consider cheeses to be adulterated if non-toxigenic *Escherichia coli* (*E. coli*) levels exceeding 10 MPN/g but less than 100 MPN/g are found in 3 or more of 5 subsamples. It is unknown whether these standards impact food safety, and the extent to which these standards will impact domestic and imported cheeses.

Purpose: To determine *E. coli* levels and compliance with 1998, 2009, and 2015 FDA CPG non-toxigenic *E. coli* criteria in tested cheese samples.

Methods: FDA's Compliance Program results from January 1, 2004 to December 31, 2006 were analyzed to determine *E. coli* levels and compliance with 1998, 2009, and 2015 FDA CPG non-toxigenic *E. coli* criteria. Data was also analyzed to determine correlations between *Listeria monocytogenes* (*L. m.*) and non-toxigenic *E. coli* levels in cheese samples.

Results: Out of 3,413 cheese samples tested for non-toxigenic *E. coli* during FY 04, 05, and 06, 2,584 (75.7%) samples contained *E. coli* levels that exceeded 10 *E. coli*/g. Of these samples, 2,287 (67.0%) exceeded 2009 regulatory guidelines of 100 *E. coli*/g. In comparison, out of 3,413 cheese samples tested, only 333 (9.8%) of tested cheese samples exceeded EU standards (<1,000 *E. coli*/g). Of these samples, only 242 (7.1%) exceeded 1998 CPG criteria (<10,000 *E. coli*/g). Mexican-style soft, semi-soft, and soft ripened cheeses were most impacted by the 2015 non-toxigenic *E. coli* standards. Cheese samples that tested positive for *L. m.* showed poor correlation to non-toxigenic *E. coli* levels. Samples that tested positive for high levels of *L. m.* had associated non-toxigenic *E. coli* levels as low as <3 *E. coli*/g.

Significance: Based upon this analysis, FDA's 2015 CPG non-toxigenic *E. coli* standards will have major impacts on domestic and imported cheeses without concomitant food safety benefits.

P2-143 Validating the Efficacy of Cleaning Procedures Used to Reduce Microbial Loads on Wooden Boards Used for Cheese Aging

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Introduction: Artisanal cheesemakers follow traditional European practices of aging cheeses on wooden boards. The Food and Drug Administration's current regulations mandate that utensils and other surfaces that contact food must be adequately cleanable and properly maintained. Development of simple methods to assess the efficacy of board cleaning and sanitizing regimens will assist cheesemakers in providing food safety assurance.

Purpose: To develop a method to assess and validate the efficacy of cleaning procedures used to reduce microbial loads on wooden boards used for cheese aging.

Methods: Six sponge samples per board (3M Sponge stick moistened with Dey-Engley Neutralizing Broth) were taken from previously used wooden boards, where a 4"x 4" area was swabbed at random. Swabs were obtained prior to and following cleaning and sanitizing procedures. The wooden boards studied were used to age three different cheese varieties: Alpine style, Blue, and Cheddar; and counts following treatments were compared to determine if cheese type influenced results. Sponges were analyzed by removing 1 ml aliquots and plating using 3M APC Petrifilm, where levels of bacteria were enumerated pre-and post-board washing and drying. ANOVA was performed to determine statistical significance ($P < 0.0001$).

Results: Analysis of 150 samples revealed average microbial loads on boards prior to washing of 6.6 log CFU/cm^2. By cheese type, counts were 5.94 log CFU/cm^2 for Alpine, 7.16 log CFU/cm^2 for Blue, and 6.93 log CFU/cm^2 for Cheddar. Cleaning regimens consisting of hot water bath (46.5**°**C 30 seconds), sanitizing with 200-250 ppm Peroxyacetic acid for 2 minutes, and manual scrubbing, resulted in counts averaging 3.37 log CFU/cm^2, 4.21 log CFU/cm^2, and 3.58 log CFU/cm^2, respectively. The cleaning regimens analyzed showed a consistent 3.75 log/cm2 reduction in count, regardless of cheese type.

Significance: These results indicate that the outlined method can be easily, reproducibly and cost-effectively used by cheese makers to validate the efficacy of wooden board cleaning/sanitizing procedures.

P2-144 Fate of *Listeria monocytogenes* in Three Types of Cheese Products Stored 42 Days at 7°C

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Introduction: The US Food Code (2013) limits storage of commercially processed non-standardized cheese products to seven days once opened when held under refrigeration. In many delis, this is not sufficient time for bulk cheese to be used completely.

Purpose: This study aimed to determine the potential for growth of *L. monocytogenes* and native populations of bacteria, yeast, and mold on cheese slices stored 42 days at 7°C.

Methods: Three LAND O'LAKES cheese products, two pasteurized process cheeses (New Yorker American and Reduced Sodium Deli American) and one natural cheese (Reduced Fat Swiss), were sliced using a Hobart model 2912 deli slicer, surface inoculated with a *L. monocytogenes* cocktail (ATCC 19115, 19118, 43257, 51779, and 51782) at 3.7 ± 0.1 log CFU/g, and air-dried 15 min. The slices were then folded and placed in a Whirl-pak bag. Periodically during 42 days storage at 7°C, three slices of each product were plated on Modified Oxford Agar to enumerate *Listeria*. Similarly, uninoculated cheese slices were plated on Plate Count Agar and Standard Methods Agar w/ Chloramphenicol to enumerate mesophilic aerobic bacteria, yeast and mold, respectively. Findings were analyzed using 2-sample *t*-test with equal variance (Minitab 17).

Results: After 42 days of storage at 7°C, populations of *L. monocytogenes* declined significantly ($P < 0.05$) to 3.1 ± 0.1, 3.2 ± 0.0 and 2.1 ± 0.3 log CFU/g in New Yorker American, Reduced Sodium Deli American, and Reduced Fat Swiss, respectively. Similarly, populations of native mesophilic bacteria significantly ($P < 0.05$) decreased in New Yorker American and Reduced Sodium Deli American. Bacterial counts were not performed on the Reduced Fat Swiss due to the presence of starter cultures.

Significance: This study supports the holding of opened packages of processed cheese in retail delis stored under proper refrigeration longer than the seven day limitation found in Food Code (2013).

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P2-145 Evaluating the Efficacy of Commercially Produced Protective Cultures for Controlling *Listeria monocytogenes* in Broth, Milk, and High Moisture Cheese

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❖ ***Developing Scientist Competitor***

Introduction: Emerging consumer demand for safe yet minimally processed, "clean label" foods necessitates the development of natural interventions to mitigate the threat of *Listeria monocytogenes* as contaminants in dairy products. The use of protective cultures is one promising alternative.

Purpose: The purpose of this research was to evaluate the efficacy of commercially produced protective cultures of lactic acid bacteria (LAB) in controlling *L. monocytogenes* in broth, milk, and high-moisture cheese.

Methods: Co-culture assays containing each of eight LAB cultures and *L. monocytogenes* (100:1 ratio, respectively) were conducted in Brain Heart Infusion (BHI) and de Man Rogosa Sharpe (MRS) broths incubated at 35°C for 48 h. Similar assays were conducted in UHT milk at ratios of 100:1; 1000:1; and 1,000,000:1 incubated at 35°C for 48 h or 7°C for 7 days. High-risk cheeses (pH >6, moisture >50%) surface inoculated with *L. monocytogenes* (4 log CFU/g) were subsequently inoculated with each LAB strain (6 log CFU/g) and stored at 7°C for 36 days.

Results: Co-culture treatments identified seven strains capable of reducing *L. monocytogenes* to below detectable levels within 24-48 h in MRS with and without added rennet. Only three strains inhibited *L. monocytogenes* growth in BHI over 48 h. Strain BS-10 at a ratio of 1,000,000:1 was the only treatment that inhibited growth in milk at 35°C. All strain treatments at this ratio inhibited growth in milk at 7°C. Four LAB strain treatments produced slight reductions in *L. monocytogenes* counts on cheese and inhibited growth over 36 days at 7°C when compared to controls.

Significance: This study demonstrates the efficacy of commercially produced protective cultures for use in controlling *L. monocytogenes* under varying conditions. These results serve as the basis for the identification of cultures and their combinations to control *L. monocytogenes* throughout the manufacture and storage of high-risk cheeses.

P2-146 Reduction of *Listeria innocua* on Queso Fresco and Mozzarella Cheese Using Supercritical Fluid Extraction with CO_2

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❖ ***Developing Scientist Competitor***

Introduction: Contamination of dairy products with *Listeria monocytogenes* continues to challenge the dairy industry. In the event of post-process contamination, antimicrobials have been used to prevent the growth of *L. monocytogenes* in cheeses. Alternatives to food additives are desired by the consumer and novel methods like supercritical fluid extraction with CO_2 (SFE) could potentially reduce the microbial population and enhance the safety of cheeses.

Purpose: The purpose of this study was to determine if SFE could reduce the population of *Listeria innocua* in mozzarella and queso fresco cheese.

Methods: Cheeses were inoculated with *L. innocua* (10^6 log CFU/g), incubated overnight at 4°C and then treated with SFE at two pressures and temperatures (120 bar at 40°C and 150 bar at 50°C) for 30 min. Treated and untreated samples were analyzed for *L. innocua*, coliforms, yeasts and molds, psychrotrophs, pH and water activity immediately after treatment and at 24 h. Counts were compared using ANOVA.

Results: SFE treatment at 120 bar, 40°C for 30 min decreased *L. innocua* approximately 3.0 and 3.5 log CFU/g in mozzarella and queso fresco cheeses, respectively. Increasing SFE pressure and temperature to 150 bar and 50°C, increased *L. innocua* reductions to approximately 3.78 and 5.2 log CFU/g in mozzarella and queso fresco cheeses, respectively. The water activities and pH were 0.9640 and 5.35 for treated and untreated mozzarella cheeses. The water activities were 0.9745 for treated and untreated queso fresco cheese, while the pH was 6.67 and 6.55 for untreated and treated queso fresco cheese, respectively.

Significance: This data suggest that SFE could potentially be used to reduce *L. monocytogenes* in cheeses without negatively impacting product quality.

P2-147 Using MilliporeSigma Milliflex Quantum to Rapidly Detect and Enumerate Spoiler Microorganisms from Beer Mixes with a Low or 0% Alcohol Content to Save Time and Costs

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Introduction: The Spanish brewing industry required faster test results of beer samples.

Purpose: Traditional methods require a total of nine days for detection & enumeration. The goal was to reduce time to results while maintaining the qualitative and quantitative accuracy of microbial analysis.

Methods: A variety of rapid technologies have been evaluated and fluorescence technology selected as most suitable for detecting spoiler microorganisms in beer mixes with a low or 0% alcohol content. Validation has been performed according to ISO 16140 on 3 beers: soft drink mixes in a comparative study using a rapid method of membrane filtration and fluorescence staining and the reference method of Wallenstein agar incubated at 27°C for 9 days. Three microorganisms were tested (*Zygosacharomyces bailii*, *Dekkera anomala*, *Dekkera bruxellensis*). Six bottles of each product were spiked with the 3 microorganisms (10 CFU/filtered volume) and incubated for 3 days at 27°C on Wallenstein agar, followed by staining and read out for the rapid method and re-incubation for another 3 days to compare results with reference method after 6 days. For verification 15 replicates/ product were analyzed with both methods. As results might be absent or near 1 CFU/bottle, a number of positive controls were prepared.

Results: Validation results show a recovery rate above 70%, efficacy of 100% and a relative sensitivity and specificity of 100% stating that both methods are equivalent. Verification results show efficacy of 90%, relative sensitivity and specificity of 86% and 93%, a recovery/accuracy level of above 70% and a precision similar to the traditional method.

Significance: The validation study confirms that MilliporeSigma Milliflex Quantum can reduce the time to result to half or one third of the time required by the standard method. Faster results allow beverage manufactures to react faster and reduce risk of quality and safety issues.

Poster

P2-148 Withdrawn

P2-149 Distribution of Ethanol-resistant Lactic Acid Bacteria Present in Wineries of Queretaro, Mexico

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Introduction: Wine quality is strongly affected by autochthonous microorganisms present in the field and/or cellar equipment, especially those able to grow in wine. Some lactic acid bacteria (LAB) species have been associated to wine spoilage and biogenic amines production, while others produce the malolactic fermentation (a desirable process). In the emerging wine region of Queretaro, México the LAB species present, their reservoirs and their possible wine quality impact are unknown.

Purpose: To identify the ethanol-resistant LAB species from strategic materials of wineries established in Queretaro.

Methods: LAB were isolated using three culture media, from must, wine and barrel/filters samples collected in four wineries of Queretaro. Tolerance to ethanol (10-13%) was assessed in a synthetic wine media containing SO_2 (30 mg L^{-1}) and a pH level of 3.5 using a Bioscreen (72 h, 30°C), those strains resistant to at least 10% ethanol were identified using species-specific primers in a Multiplex-PCR.

Results: From 61 samples 822 LAB isolates were recovered, 14% (119) were resistant to 10% ethanol. Five species were identified: *Oenococcus oeni* and *Lactobacillus plantarum*, which can be used as starter cultures; *Pediococcus parvulus, Lactobacillus hilgardii* and *Lactobacillus brevis* which have been associated to wine spoilage and biogenic amines production. The highest diversity was found in wine samples, and barrels were perceived as hazardous microbial reservoirs. With 13% ethanol, almost all species (excepting *O. oeni*) decreased the number of resistant isolates in about 50%, being spoilage species (*Lactobacillus.* spp. and *P. parvulus*) the most affected ones.

Significance: The results obtained lead to develop strategies to control spoilage microorganisms and detect possible autochthonous starter cultures in order to improve the quality of the local wines.

P2-150 Evolution of Mass Spectrometry in Laboratory Testing of Biothreat Agents

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Introduction: Clinical, agricultural and public health laboratories (PHL) screen thousands of samples daily for bacterial agents and toxins. Sample preparation, processing and analysis using conventional microbiological methods can range from days to weeks for pathogen identification. Two agents of high concern are Botulinum neurotoxin (BoNT) which cause the disease known as botulism by inhibiting neurotransmitter release at the neuromuscular junction and ricin toxin which inhibits protein synthesis

Purpose: Both methods require extensive and cumbersome processing leading to time consuming testing as negative results can take up to four days to identify. As the number of specimens has increased in laboratories, there exists a great need to quickly identify sources of exposure which has led our laboratory to explore the use of Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF/MS).

Methods: An endopeptidase-based mass spectrometry method, which was developed by the CDC, has been transitioned to be used with the Bruker Daltonics MALDI-TOF Biotyper. This MS method utilizes the endoproteinase activity of the toxin to identify all BoNT types (A-G) with a MALDI-TOF/MS by cleaving peptides at specific sites. Each toxin is identified by the mass-to-charge ratios of these fragmented peptides for BoNT and the depurination of a RNA substrate for ricin toxin.

Results: For each assay, BoNT and ricin toxins were spiked into over ten different food matrices to assess detection capability. Assay sensitivity for BoNT types A, B, E, and F ranged from 0.3 to 25 MLD_{50}. Active ricin toxin was detected as low as 5 mg/ml.

Significance: Our findings support identification of BoNT and ricin toxin using the mass spectrometry-based method, replacing the need for performing traditional costly, time-consuming methods. Furthermore, this method eliminates the need to use animals for laboratory testing.

P2-151 Attachment, Growth and Persistence of *Cronobacter* on Granular Activated Carbon Filters

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Introduction: Several *Cronobacter* outbreaks have implicated contaminated drinking water. This prompted us to assess whether activated carbon water filters commonly used in homes could serve as a potential source of *Cronobacter*. Past research has suggested that such filters can serve as potential source of bacteria in the home environment if used in conjunction with water with excess organic loads. Accordingly, the attachment, growth and persistence of *Cronobacter* in granular activated carbon (GAC) filter was investigated to assess the impact of GAC on the microbial quality of the water produced.

Purpose: This study tested the hypothesis that GAC filters could serve as a reservoir for *Cronobacter* by trapping low levels of nutrient upon which the organism could grow.

Methods: A simulated water filter system was installed by filling glass tubes with sterile GAC, followed by sterile water with a dilute carbon source (peptone) flowing through the column at a rate of 1 liter/day. Carbon columns were inoculated with 10^4 CFU of *Cronobacter* on the surface, and the effluent monitored for Cronobacter levels. During a second phase, commercial faucet filters will be distributed to households for 4-month use. Used filters were backwashed with sterile peptone water, and analyzed for *Cronobacter*, total aerobic plate count, coliform bacteria and *Enterobacteriaceae*.

Results: *Cronobacter* colonized the simulated GAC and grew when provided minimal levels of nutrients, shedding up to 10^7 CFU/ml in the effluent water after 2 days. Most *Cronobacter* colonized on the upper part of the column. Backwashed used filters used in home settings yielded presumptive *Cronobacter* spp., *Escherichia coli*, *Pseudomonas* and other waterborne bacteria.

Significance: *Cronobacter* and other bacteria are able to colonize on granular activated carbon filter, penetrate the treatment barrier and release into water effluent, introducing a safety risk of waterborne pathogens, and potentially contaminating the kitchen environment. These results suggest that the use of these filters should be considered a potential source of *Cronobacter* and other foodborne pathogens under certain circumstances.

P2-152 Pathogenic Parasite Accumulation in Environmental Biofilms in an Endemic Location

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Poster

Introduction: Aquatic biofilms trap waterborne protozoan parasites, some of these parasites cause significant disease in humans. These parasites can be released from the biofilm and cause contamination in subsequent water usage. *Cryptosporidium*, *Giardia*, and *Cyclospora* are considered among the main protozoan parasites of concern for food production.

Purpose: The purpose of this study was to locate sources within biofilms of protozoan parasites in water used for agriculture.

Methods: Biofilms were collected from agricultural water surfaces in an endemic area for *Cyclospora cayetanensis* and analyzed for the presence of protozoan parasites. *Cryptosporidium* and *Cyclospora* were detected using nested PCR-RFLP using the 18S ribosomal RNA as a target gene. *Cyclospora* was also observed using light and epifluorescence microscopy. *Cryptosporidium* and *Giardia* were detected using immunofluorescence tools (IFA).

Results: Analysis of 64 samples collected in an endemic area during the low season period using nested PCR (nPCR) for *Cyclospora* resulted in 23 presumptive *Cyclospora*-positive samples. By RFLP one was confirmed positive. Two samples were positive for *Cryptosporidium* sp. by nPCR and four by IFA. *Giardia* sp. was identified in 24 samples by IFA.

Significance: This study demonstrates that biofilms on surface water used for agricultural purposes can serve as a reservoir for protozoan parasites and could be a potential source for foodborne parasitic infections.

P2-153 Biofilm Formation of Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) on Equipment Surfaces

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) serotype O157:H7 has been the most commonly recognized STEC serotype responsible for foodborne outbreaks in the US. Numerous outbreaks associated with non-O157 serotypes have also been reported due to consumption of contaminated food. The six major serotypes, referred to as "the big six," can attach and form biofilms on food contact surfaces, and subsequently contaminate food at the processing facility.

Purpose: The purpose of this study was to compare different non-O157 STEC for curli expression and biofilm formation.

Methods: Biofilm formation of 12 non-O157 serotypes (O26, O45, O103, O111, O121, O145) was determined using a CDC Biofilm Reactor assay. Stainless steel, PTFE and polycarbonate coupons (n=180) were used in the reactor containing 400 ml of 10% nutrient broth (NB) which had been inoculated with 1 ml of overnight culture. The reactor was set with a continuous flow of 10% NB and shear forces of a swirling paddle for 24 h. After 24 h, coupons were scraped off to remove attached non-O157 and populations of non-O157 were determined by plating procedure. Curli expression of strains was identified by their affinity to Congo red (CR) dye.

Results: Biofilm formation of non-O157 serotypes varied with material surface and strain. Most strains were recovered at significantly higher levels on PTFE and polycarbonate surfaces compared to populations recovered on stainless steel surfaces. *E. coli* O26 strain 3629 recovered from stainless steel, polycarbonate and PTFE (7.06 to 7.44, log CFU/cm^2) were significantly higher than *E. coli* O145 strain 3419 recovered from corresponding surfaces. In general, curli-expressing strains formed stronger biofilms on material surfaces.

Significance: Biofilm formation of non-O157 serotypes varies among EHEC serotypes and is influenced by the equipment surface. Understanding biofilm mechanisms will be helpful in evaluation of contamination risk and intervention strategies to prevent contamination of foods.

P2-154 Microbial Reduction of Dried Laver (*Porphyra tenera*) and Identification of Resistant Bacteria after Electron Beam Treatment

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❖ ***Developing Scientist Competitor***

Introduction: Laver (*Porphyra tenera*) is one of the most favorite brown seaweeds in Korea, containing essential amino acids, vitamins, and fiber. Since dried laver is contaminated by manufacturing process, new microbial reduction method is required to ensuring its safety.

Purpose: This study investigated the effect of electron beam (EB) treatment on the microbial reduction of dried laver and identified EB–resistant bacteria from the treated dried laver.

Methods: After EB treatment of 4 kGy and 7 kGy, the numbers of total bacteria and EB-resistant bacteria were measured using tryptic soy agar (TSA) and mannitol salt agar (MSA), respectively. The morphological and biochemical characteristics of each isolated EB–resistant bacterium was investigated using gram-staining, catalase test and salt tolerance test. These bacteria were identified by using 16S rDNA sequence analysis.

Results: Compared to the number of total bacteria in the control group, the total bacterial number after EB treatment of 4 kGy and 7 kGy was significantly decreased to (5.4 ± 0.5) × 10^4 CFU/g and (1.1 ± 0.6) × 10^4 CFU/g, respectively. With the increases of EB dosage, the number of red colony was almost the same whereas yellow colony was significantly decreased to (3.3 ± 1.2) × 10^3 CFU/g and 0 CFU/g for 4 kGy and 7 kGy, respectively. All red and yellow colonies were gram-positive cocci, catalase–positive and resistant to TSA media containing 5% NaCl. From the result of 16S rDNA sequence analysis, yellow and red colonies were identified as either *Micrococcus flavus* or *M. luteus* with 99% similarity for yellow colony, and *Deinococcus proteolyticus* and *D. piscis* with 99% and 97% similarity for red colony, respectively.

Significance: This study demonstrated that EB treatment was an effective method in the reduction of total bacterial number except for some of EB-resistant bacteria.

P2-155 Norovirus Prevalence and Persistence on Environmental Surfaces during Outbreaks in Long-term Care Facilities

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❖ ***Developing Scientist Competitor***

Introduction: In the US over 60% of norovirus outbreaks occur in long-term care facilities (LTCFs), making them a reasonable target for studying the role of environmental surfaces in transmission of norovirus during outbreaks.

Purpose: To evaluate environmental surface contamination during norovirus outbreaks at LTCFs.

Methods: Macrofoam swabs were used on hard surfaces in ill patient rooms and from common areas in 4 metro Atlanta LTCFs during norovirus outbreaks. Sampling was conducted as soon as each outbreak was reported (phase 1) and after the outbreak was concluded (phase 2). A total of 172 samples were collected. Viral RNA was extracted from swabs and further concentrated. Norovirus was detected by realtime RT-PCR and positive

samples were genotyped after conventional RT-PCR and sequencing. Stool samples were collected from all outbreaks and tested as part of normal outbreak investigation.

Results: Of the 88 swabs collected during phase 1, 29 (33%) tested positive for GII norovirus while 28 (32%) of 88 swabs collected at phase 2. Positive samples included door knobs, hand rails, tables, toilets, and other surfaces in patient rooms and common areas. Toilets seats in ill resident rooms were the most frequently identified positive surfaces. Viral loads ranged from 1.35 to 5.21 log gc per sampling site. Identical GII.4 Sydney sequences were identified in 7 swab and stool samples from each outbreak.

Significance: During norovirus outbreaks at LTCFs environmental surfaces become contaminated with high levels of norovirus and these surfaces remain contaminated beyond the duration of the outbreak. Review of current surface disinfection procedures may be needed to help reduce norovirus transmission via contaminated surfaces. Similar vigilance is needed for surfaces that are used for preparation of foods.

P2-156 Effects of X-Ray Irradiation on Murine Norovirus-1 in Salmon Sushi

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Introduction*:* Seafood products are among the most associated food with norovirus outbreaks. The FDA has approved ionizing radiation of up to a maximum of 5.5 kGy as a pathogen intervention strategy in fresh seafood products.

Purpose: The purpose of this investigation was to study the effect of X-ray treatments on murine norovirus-1 (MNV-1) in salmon sushi.

Methods: Salmon sushi samples (25 g) were inoculated with 100 µl MNV-1 virus stock solution. Then the inoculated samples were air dried for 30 min at 22°C in biosafety cabinet. Inoculated salmon sushi samples were placed into sterilized bags prior to X-ray treatments. Salmon sushi samples were treated with 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 KGy X-ray.

Results: The log PFU g^{-1} was significantly (P<0.05) reduced from 5.2+0.2 to 4.7+0.6, 4.2+0.3, 3.4+0.5, and 3.0+0.6 after treatment with 1.0, 2.0, 3.0, and 4.0 kGy X-ray, respectively, in salmon sushi. Treatment with 4.0 kGy X-ray achieved a 2.2-log PFU g^{-1} reduction. Treatment with 5.0 kGy X-ray reduced the population of MNV-1 to below the detection limit (<2.0 log PFU g^{-1}).

Significance: Our results indicate that the treatment with X-ray could be a suitable non-thermal processing option to the seafood sushi industry.

P2-157 Binding of Human Norovirus to a Broadly Reactive Bacterial Ligand

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Introduction: Human norovirus is the leading cause of foodborne gastroenteritis. Recent studies suggest bacteria may influence infectivity, persistence and replication of this virus, yet this phenomenon is poorly understood.

Purpose: With a focus on detection applications, this study sought to identify a broadly reactive bacterial ligand that might be used to facilitate norovirus capture and/or detection.

Methods: Seven previously characterized bacterial isolates (2 ATCC strains; 5 fecal isolates obtained from norovirus-positive stool samples) were used in this study. Isolates were grown in minimal media overnight and sonicated. These whole bacterial lysates were examined for three characteristics (1) histo-blood group antigen (HBGA) activity; (2) lectin binding; and (3) human norovirus binding. Western blots determined which bacterial glycoproteins bound the widest variety of viral strains and identified potential residues facilitating virus-bacteria interactions. Glycoproteins binding multiple human norovirus strains were isolated, purified and further analyzed using LC-MS.

Results: All of the bacterial species tested possessed various levels of HBGAs (relative densities: AB 1.0-15.8; B 0.5-5.1; H 0-0.8; Le^a 0-2.1; Le^b 0-1.3; Le^y 0-0.7). Lectin binding narrowed the bacterial residues to specific sugars: N-acetyl galactosamine, relative density 0.2-5.7; α-D-mannose/glucose, relative density 0-10.7; α-L-fucose, relative density 0.8-4.9; and α-D-galactose, relative density 0.0-4.7. A virus overlay confirmed that select human norovirus strains bound these sugar residues with relative densities of: GI.6, 0-0.8; GII.4, 0-0.2; and GII.17, 0.0-0.8. Six bacterial strains bound the GII norovirus strains, via glycoproteins of 140 kDa, 35 kDa, and 17 kDa. A 35 kDa glycoprotein from a *Bacillus* fecal isolate bound the GI and GII human norovirus strains tested.

Significance: This is the first study to identify specific bacterial residues possessing human norovirus binding potential. With further development, these molecules may be exploited as potential ligands for human norovirus capture and/or detection.

P2-158 Retention of Tulane Virus and Murine Norovirus by Zero-valent Iron Treated by Various Elution Buffers

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Introduction: Removal of Tulane virus (TV) and murine norovirus (MNV) from water filtered through sand with zero-valent iron (ZVI) has been demonstrated. ZVI has been proposed to interact with viruses through electrostatic forces.

Purpose: Reversibility of ZVI retention of TV and MNV from water was investigated by viral elution with various buffers.

Methods: TV and MNV in deionized water (0.5 ml, 4 and 5 log PFU/ml, respectively) were exposed to sand and ZVI:sand (1:1) (1 cm^3) in tubes for 30 minutes at 21°C in duplicate. Inoculants were aspirated; sand and ZVI:sand were agitated for 30 minutes in water or elution buffer (citrate buffer, pH 4 and 7, and virus elution buffer, pH 9.5, with and without added 1M NaCl). TV and MNV in the eluates were tested by plaque assay in LLCMK2 and RAW 264.7 cells, respectively. Positive and negative controls were included for eluents and water rinsates of sand and ZVI:sand.

Results: Recovery of TV from treated water was 3.7 and 2.6 log PFU/ml from sand and ZVI:sand, respectively. Eluted TV averaged 3.4 log PFU/ml (sand) and 1.9 log PFU/ml (ZVI:sand). Recovery of MNV from treated water was 4.6 and 3.6 log PFU/ml from sand and ZVI:sand, respectively. Eluted MNV averaged 4.3 log PFU/ml (sand) and 3.1 log PFU/ml (ZVI:sand). Virus retention by ZVI:sand was significantly (P<0.05) greater than sand alone. There were no significant differences (P>0.05) in elution of infectious virus.

Significance: Comparable recovery of TV and MNV by water and buffers suggests viruses are inactivated by ZVI or viral dissociation from ZVI requires disruption of additional interactions by strategies other than those normally used to recover virus from environmental samples. These data further support the potential efficacy of ZVI for remediation of viral-contaminated water.

P2-159 Inactivation of Murine Norovirus (MNV-1) on Strawberries by Pulsed Light (PL)

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Poster

Introduction: Multiple foodborne illness outbreaks associated with strawberries have raised safety concerns about berries and berry products in recent years. Human norovirus was the leading causative agent in fresh produce outbreaks. Pulsed light (PL) is an emerging non-thermal 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 murine norovirus (MNV-1), a surrogate for human norovirus, on strawberries using PL.

Methods: Fresh strawberries were spot-inoculated with MNV-1 to a level of approximately 10^5 PFU/g on the surface and treated with PL using Xenon Steripulse XL-3000TM system. Samples were exposed to PL at a rate of 3 to 100 pulses/s for up to 120 s, and the distances between quartz window and the treatment spots were from 8.3 to 13.3 cm. After treatment, viruses were extracted and recovered from the samples and quantified by viral plaque assay.

Results: The initial inoculum level was 4.8 ± 0.3 log PFU/g of sample. PL treatment was effective inactivating MNV-1 on strawberries. For different duration from 30 to 120 s, various levels of reductions were achieved. For example, at the distance of 10.8 cm, PL treatment for 30, 60, 90 and 120 s resulted in 1.4 ± 0.3, 1.5 ± 0.1, 2.4 ± 0.4 and 3.3 ± 0.4 log PFU/g reductions on strawberries, respectively.

Significance: The present study demonstrated that PL treatment was an effective intervention method to inactivate virus on strawberries and could be applied to improve the microbial safety of fresh strawberries.

P2-160 Application of High Pressure Processing on Frozen Strawberries to Inactivate Murine Norovirus

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❖ ***Developing Scientist Competitor***

Introduction: Frozen strawberries are often used as ingredients in food products such as salads, desserts and yogurt, or packaged with other kinds of frozen berries in wholesale markets. Human norovirus (HuNoV) outbreaks have occurred in recent years over the world due to the consumption of contaminated frozen strawberry products. High pressure processing (HPP) has been recognized as a novel processing technology to preserve the natural flavor and raw character of fresh produce.

Purpose: The objective of this study was to apply HPP to inactivate murine norovirus (MNV-1), a surrogate for the uncultivable HuNoV, on whole frozen strawberries.

Methods: Fresh strawberries were spot-inoculated with approximately PFU/g of MNV-1 on the surface and frozen at -20℃ for one day. The frozen strawberries were then treated at 250, 300, 350 and 400 MPa for 3 minutes with the initial start temperature of 4℃. Plaque assay analysis was performed to assess the viral inactivation results.

Results: The initial inoculum level was 4.0±0.1 log PFU/g of strawberry samples. Frozen storage did not have significant reductions of MNV-1 whereas HPP effectively inactivated the viability of MNV-1. The viral reduction level for whole frozen strawberries increased as treatment pressure increases. After high pressure treatments at 250, 300 and 350 MPa, the reduction of MNV-1 was 1.2±0.5, 2.2±0.3 and 2.4±0.4 log PFU/g, respectively. At 400 MPa for 3 minutes, MNV-1 was inactivated to a level beyond the detection limits of the plaque assay and the level of inactivation was estimated to be more than 3.6 log PFU/g.

Significance: By using the surrogate virus (MNV-1) to test the feasibility of inactivating HuNoV, our study provided insight for fresh and frozen produce industry to apply high pressure on strawberries as an effective processing tool to mitigate the risk of HuNoV.

P2-161 Isolation and Characterization of Bacteriophages Targeting Non-O157 Shiga-toxigenic *Escherichia coli*

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❖ ***Developing Scientist Competitor***

Introduction: Bacteriophages have been extensively beneficial in human and veterinary medicine, however, their application in foods is still limited. The recent FDA-approval of *Listeria monocytogenes*-specific-phage in meat and poultry has encouraged research into other phage-based products for food industry. One such application is their use as biocontrol agents against Shiga-toxigenic *Escherichia coli* (STEC). However, their application in foods requires them to be stable in a food matrix, especially in ready-to-eat and cooked products.

Purpose: Isolation and characterization of bacteriophages specifically targeting non-O157 Shiga-toxigenic *Escherichia coli.*

Methods: Bacteriophages, isolated from cattle operations in Oklahoma, were tested for lytic activity against 6 non-O157 STEC serogroups (O121, O145, O111, O103, O26, O45) using spot-on-lawn assay. Bacteriophages were purified and their morphology determined using transmission electron microscope (TEM). Thermal resistance was determined between 40-90°C for 60 minutes with 10-minute-sampling-intervals. Stability at various pH ranges (1-11) was analyzed at 37°C with sampling times between 1-24 h.

Results: Several bacteriophages (*n*=44) were isolated that exhibited inhibition towards non-O157 STEC. Except for the O45-specific phage, all other phages showed multiple-target-specificity. For example, O26-specific phage also showed lytic activity against O103 and O145 STECs. The TEM micrographs placed the O45, O26, O103, O145, and O121 phages into *Myoviridae* family except one O45-phage in *Tectiviridae* and one each of O103 and O26-phage in *Siphoviridae* family. The O111-phages belonged to the *Siphoviridae* family. Bacteriophages were very stable at 40-60°C; some (O111, O103, O45) lost activity at 70°C after 30 minutes; and all lost activity at 90°C after 10 minutes, except O121 and O26 phages. Phages had high infective ability between pH range 5.0-11.0 with the exception of O26-phage, showing infective ability at pH 1.0 and 2.0 for 2 h.

Significance: Bacteriophages showing high lytic activity towards non-O157 STECs, that are also pH and thermal stable, could serve as bio-preservatives in the food industry.

P2-162 The Role of *Pseudomonas aeruginosa* DesB on Stress Responses

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❖ ***Developing Scientist Competitor***

Introduction: *Pseudomonas aeruginosa,* one of food spoilage bacteria, can be exposed to various environmental stresses, especially acid or NaCl in food environments. Bacteria can adapt to these stresses by adjusting the ratio of saturated fatty acid (SFA) and unsaturated fatty acid (UFA) to

maintain the membrane fluidity. Previously, it was reported that heterologous expression of *Arabidopsis* fatty acid desaturase (FAD2) increased the resistance to NaCl in *Saccharomyces cerevisiae*.

Purpose: The objective of this study was to investigate the role of aerobic desaturase, DesB in stress responses of *P. aeruginosa*.

Methods: *P. aeruginosa* PAO1 (wild type; WT) and its derived mutants (harboring a mutation in *desB*, *desT*, *desA*, or *fabA*) were used to determine if the genes, associated with UFA synthesis, are responsible for the adaptation to hostile conditions. The cultures were exposed to oxidative, osmotic or acidic stress, and the bacterial growth was observed. To elucidate the underlying mechanism of DesB on stress response, transcriptional levels of WT and *desB* mutant were compared using qRT-PCR.

Results: The growth of *P. aeruginosa* WT and its derived mutants displayed no difference regardless of exposure to oxidative or acidic condition. Also, the bacterial strains showed similar growth in the presence of 0.2 M NaCl, whereas their growth was inhibited slightly or severely under the exposure of 0.5 or 1.0 M NaCl, respectively. More importantly, *desB* mutant exhibited more impaired growth than WT and other mutants under these high concentrations of NaCl, suggesting the role of DesB on osmotic stress. Transcriptional analysis revealed that genes associated with osmoprotectant synthesis were highly expressed in response to NaCl in WT, but rarely expressed in *desB* mutant.

Significance: The results demonstrate that *P. aeruginosa* DesB has an important role in resistance and adaptability to osmotic stress through affecting the expression of osmoprotectant synthesis genes.

P2-163 Natural Occurrence of HT-2 and T-2 Toxin and Its Production of *F. armeniacum* Isolated in Rice

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Introduction: T-2 toxin and HT-2 toxin are mycotoxins of the group trichothecenes type A produced by the *Fusarium* genus which are commonly found in various cereal crops. T-2-and HT-2 toxin often occur together in infected cereals and their contamination of human foods and animal feeds is a continuing worldwide problem.

Purpose: The purpose of this study was to illustrate the mycotoxin production of causal fungus associated with natural occurrence of T-2 and HT-2 toxin in rice.

Methods: Identification and incidence of T-2 toxin producing *Fusarium armeniacum* isolates and natural occurrence of T-2 toxin and HT-2 toxin in 2012–2013 rice grains collected in Korea. Mycotoxins produced by the *Fusarium* isolates and rice grain samples were detected with LC-MS.

Results: HT-2 toxin only was found in polished rice sample with 0.02 ppb (*n*=1) in 2012 and unpolished 0.1 ppb (*n*=4), brown 0.1-0.2 ppb (*n*=2), polished 0.1-0.2 ppb (*n*=3) in 2013. *F. armeniacum* isolated from grain samples contaminated with HT-2 toxin. In total 526 Fusarium isolates, *F. armeniacum* was 50 isolates. To investigate mycotoxin production of these isolates (*n*=15) were cultured in rice grain for 21days. T-2 and HT-2 toxin of these isolates were detected with 173.6-414.9 ppm, 42.0–76.0 ppm.

Significance: Our study suggest that trichothecene A type in rice will be problem depend on environmental condition in Korea. The further study on monitoring *Fusarium* contamination and natural occurrence of mycotoxin should be continued.

P2-164 Investigating the Dynamic Flow of *Bacillus* Physiological States from Spore to Cell Multiplication

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✦ ***Undergraduate Student Award Competitor***

***Introduction*:** Spore-forming bacteria in food are a major cause of food poisoning or food spoilage, leading to a heavy burden. Heat treatment is the main hurdle used to eliminate spores in foods. The spores can resist to such treatments and several physiological stages of germination can be observed: dormant spores, germinated spores and vegetative cells.

Purpose: The aim of this study is to follow by flow cytometry the fate of spores after specific heat-treatments and recovery in sub-optimal conditions. Especially, the dynamic of the physiological states leading the spore to give a vegetative cell was studied.

Methods *:* The spores of *Bacillus weihenstephanensis* KBAB4 and *Bacillus licheniformis* AD978 were obtained at optimal and suboptimal growth temperatures. After heat-treatments allowing a ten-fold reduction, spore recovery in nutritive broth was investigated for different pHs and temperatures distributed along the growth domain (5-40°C for *B. weihenstephanensis*, 15-60°C for *B. licheniformis* and pH 4.0-8.0 for both strains). The germination and growth recovery were monitored over time using flow cytometry, taking into account cell size and permeability (Syto9 staining) and respiratory activity (CTC staining).

Results: Different physiological stages were efficiently evidenced: refractive spores, germinated spores, outgrowing cells and vegetative cells. In optimal conditions, most cells evolved rapidly towards multiplication. Recovery of heat-treated spores was slower at suboptimal pH and temperature than at optimal pH and optimal temperature, and a significantly lower proportion of spores successfully formed vegetative cells. Although large proportion of spores germinated and showed some early signs of potential growth after a heat-treatment, only a few were actually able to form a colony on agar plates. Monitoring spore recovery using flow cytometry is a powerful method, allowing an exploration of the individual evolution of cells among populations. The number of analysed cells is potentially high (over 200 000 in the present work), offering an accurate estimation of variability in individual cell development within populations.

Significance: A mathematical probabilistic model has been developed to describe the dynamic aspects of heat-treated spore germination and growth restoration and allow the estimation of the proportion of each physiological stage regarding incubation temperature and pH, and the duration of incubation. This can lead to a better prediction of spore forming bacteria development in foods.

P2-165 Total Polyphenols, Antioxidant Activity and Antibacterial Effect of Nine Cultivars of Cactus Pear (*Opuntia* spp.) and Their By-products

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Introduction: Nopal cactus (*Opuntia* spp.) is an important resource of semi-arid zones of the world, where fruits and stems are used as food or forage, depending on the variety or ripeness of the cladode. *Opuntia* genus has a good capacity of adaptation, and comprises a large number of species with variable phytochemical composition. About 20% of the cladodes are by-products (modified leaves or thorns) that are usually discarded.

Purpose: To evaluate the antioxidant and antibacterial potential of 9 nopal cultivars and their by-products.

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Methods: Cladodes and by-products from 9 nopal cultivars were washed, dried and macerated in ethanol, evaporated and the extract resuspended in water. The antimicrobial activity against *V. cholerae* and *C. perfringens* was determined. Preliminary analyses were conducted by the well diffusion method. Minimal bactericidal concentrations (MBCs) were determined using the broth microdilution method. Quantification of total phenols and flavonoids, and antioxidant capacity, as TEAC and inhibition percentage of DPPH radical, were performed by spectrophotometric methods.

Results: All extracts showed antioxidant and antibacterial activity. Extracts of cladodes and by-products showed similar MBC (around 7.5 mg/ml) for *C. perfringens* and (4.0 mg/ml) for *V. cholerae*. The cladode by-products showed higher antioxidant capacity (DPPH and TEAC tests) when compared with cladodes. High concentrations of phenols and flavonoids were also found in the by-products analyzed (ranges from 91.23 to 853.78 mg GAE/g DW and from 17.10 to 39.42 mg QE/g DW).

Significance: This study demonstrated the antimicrobial activity of nopal cactus byproducts with a higher content of phenols and flavonoids, and an increased antioxidant activity compared with cladodes.

P2-166 Electron Beam Processing Improves the Microbiological Safety and Retains the Sensory Qualities of Alfalfa Sprouts

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Introduction: There has been a significant increase in the consumption of fresh produce in the US. This increased consumption puts consumers at risk of pathogen exposure since there is no pathogen kill step for sprouts. The hypothesis was that electron beam (eBeam) technology can significantly reduce pathogen exposure via alfalfa sprouts without impairing sensory qualities.

Purpose: The objective was to demonstrate the effectiveness of eBeam (at a FDA approved dose of ≤ 1 kGy) as a non-thermal pathogen kill technology to improve alfalfa sprouts' microbiological quality without impairing its sensory qualities.

Methods: Alfalfa sprout samples were inoculated with a cocktail of non-O157 STEC strains and processed using the eBeam technology (dose ≤ 1 kGy). The eBeam treated and control samples were compared in terms of the reduction of natural bioburden as well as the levels of the inoculated non O157 STEC pathogens. The treated and control samples were also compared in terms of texture, color, and electrolyte leakage for sensory attributes.

Results: eBeam beam processing of alfalfa sprouts at doses ≤ 1 kGy achieved a 4-log reduction of the non-O157 STEC strains. The natural bacterial and fungal bioburden levels were reduced by 2.2 and 2.1 logs, respectively, over a 21-day refrigerated storage. There was no significant ($P \leq 0.05$) difference in texture, color, or electrolyte leakage between eBeam treated and (untreated) control samples.

Significance: eBeam treatment of alfalfa sprouts at doses (i.e., ≤ 1 kGy), an FDA approved technology to extend shelf life has a major collateral benefit of achieving a 4-log reduction of a key bacterial pathogen associated with alfalfa sprouts without impairing the sensory qualities.

P2-167 UV-C Sensitivity of Pathogenic and Attenuated *E. coli* O157:H7 Strains in Relationship with Inactivation Mechanism

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Introduction: UV-C light, as a postharvest intervention, is simple to implement and inexpensive for commercial applications. Non-pathogenic/attenuated surrogates of pathogenic bacteria should be used to conduct trials on a large scale or at a commercial setting, due to the difficulty of containment and decontamination.

Purpose: The objectives of this study were to compare the UV-C sensitivity of various pathogenic and attenuated *E. coli* O157:H7 strains, and to investigate the mechanism of UV-C disinfection.

Methods: Planktonic cells of six pathogenic *E. coli* O157:H7 strains associated with recent outbreaks of foodborne illnesses, and four attenuated *E. coli* O157:H7 strains were exposed to varying doses (0-913 mJ/cm^2) of UV-C. The mechanism of UV-C damage on two pathogenic *E. coli* strains with different UV-C sensitivities was evaluated using real time PCR assays with ethidium monoazide (EMA) pre-treatment.

Results: Results showed that the attenuated *E. coli* strains had similar overall UV-C sensitivity as the pathogenic counterparts. The UV-C inhibition on the PCR amplification of DNA correlated well with UV-C dose, as indicated by the cycle threshold (Ct), regardless of EMA pre-treatment. The Ct values increased linearly (R^2=0.98) with increasing UV-C doses in the 0-203 mJ/cm^2 dose range. The difference in UV-C sensitivity between strains RM6535 and EDL933 (i.e., the most UV-C sensitive and resistant pathogenic strains, respectively) was reflected by increases in Ct values, viz., increases in Ct values as a function of UV-C dose were significantly ($P<0.05$) higher for RM6535 than for EDL 933. EMA-PCR analysis indicated that the cell membrane was affected only at high doses when UV-C inactivated more than 6 log CFU/ml of bacteria.

Significance: Overall, our results suggest that the attenuated *E. coli* strains, on average, had similar sensitivities to UV-C as the pathogenic strains. Furthermore, real-time PCR assays can be used to assess DNA damage caused by UV-C.

P2-168 Effects of Microwave Power Level and Time on *Escherichia coli* P511 in Microwavable Foods

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Introduction: Microwave heating utilizes electromagnetic waves of certain frequencies to produce heat energy in food materials while minimizing thermal degradation of the food components. Using proper microwave heating techniques (i.e., correct power level and time) microwave heating has potential in inhibiting foodborne pathogens.

Purpose: It is the purpose of this study to determine the survivability of *Escherichia coli* P511 after microwave treatments.

Methods: Microwavable foods were microwaved with different treatments at 270 W for 60 s, 950 W for 150 s and 300 s. Twenty-five g of the treated samples were tested for total coliform counts, aerobic plate counts, presumptive coliform test, *E. coli* and *Salmonella*. Survivability (challenge tests) of *E. coli* P511 were determined in microwavable food samples at 300, 600 and 900 W at 60, 90, 120, 150 and 180 seconds.

Results: Total bacterial count for all microwavable food samples were below detection limit. Challenge tests showed that the microbial count of *E. coli* P511 can be reduced from an initial 9.47 log CFU/g to 2.67 log CFU/g at 600 W for 120 s with an average internal temperature of 60.09±0.82°C. *E. coli* counts were reduced to below detection limit when treated at 600 W for 150 s and 900 W for 90 s with an average internal temperature of 68.20±2.92°C and 65.44±3.56°C. Microwave power level at 300 W and 180 s were not sufficient to inhibit the growth of *E. coli* and the average internal temperature was 52.21±3.63°C.

Significance: Microwave heating show potential in decontaminating microwavable food at high power level and short treatment time and can be applied as part of the hurdle technology in food industry to inhibit foodborne pathogens.

P2-169 Microbiological Quality of Street-vended Juices in Jeli District, Malaysia

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Introduction: Street-vended beverages are widely accepted by consumers in Malaysia due to their taste, reasonable prices, and all-time availability. Freshly prepared juices however have no process or steps to minimize foodborne pathogens if they are contaminated.

Purpose: It is the purpose of this study to determine the microbiological properties of street-vended sugarcane juice and coconut water and associated preparation surfaces sold by three selected street vendors in Jeli, Malaysia.

Methods: A total of 18 beverages and 15 swab samples with 47 direct film samples were collected from 3 stalls in Jeli district, Kelantan, Malaysia. Coliforms and *Escherichia coli* were chosen as the safety indicator and microbiological content of beverages; whereas, *Staphylococcus* load represented the hygienic practices of vendors. Selective agar was used for conventional enumeration of microbial load while Sanita-kun dehydrated medium was used for direct stamping and enumeration of *Escherichia coli* and *Staphylococcus aureus*.

Results: Sugarcane juice recorded the highest bacterial contamination at 2.63±0.01 log CFU/ml) and coconut water at 2.56±0.01 log CFU/ml from street vendor B. The sugarcane extractor from Stall B also recorded the highest total viable counts (4.13±0.01 log CFU/ml). The results indicated that 26.67% of the preparation surfaces samples have presumptive *Escherichia coli* detected whereas stall B handler's apron had the highest *Staphylococcus* count on both Baird-Parker Agar and Sanita-kun dehydrated medium at 3.08±0.01 log CFU/ml and 3.11±0.01 log CFU/ml, respectively.

Significance: Street-vendors should practice Good Hygiene Practices (GHPs) in preparation and handling of beverages for consumption. Regular hygienic assessment and monitoring of the quality of street-vended beverages are required to ensure food hygiene and avoid foodborne illnesses.

P2-170 Validation of Pasta Cooking Instructions

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Introduction: Raw flour-based products, such as pasta, typically undergo minimal processing during production and can be a source of *Salmonella* spp. Pasta products are normally sold with labelled cooking instructions. However, there is a lack of scientific literature demonstrating the lethality when following cooking instructions for dry pasta.

Purpose: The purpose of this study was to provide scientific justification that the labelled cooking instructions for dry pasta are adequate to achieve an acceptable reduction in *Salmonella* spp.

Methods: Fresh pasta dough samples were inoculated with a cocktail of *Salmonella* spp. to achieve a target level of 8-log CFU/g. Inoculated dough samples were formed into farfalle or fettuccini pasta shapes and dried in a controlled environment chamber, meeting internally established dry pasta specifications. Following drying, samples were cooked at 160, 170 or 180°F and evaluated at several time points between 0 and 25 minutes. Pasta samples were enumerated for *Salmonella* spp. survival using Tryptic Soy Agar (TSA) with an over lay of Xylose Lysine Deoxycholate (XLD) via pour plate method. Log-transformed data were fitted to a simple regression model using least squares regression to estimate *D*- and *z*-values. The validity of the model was verified using R-square values and residual analysis.

Results: The *D*-values were 3.95, 2.10, and 1.62 min for farfalle pasta, and 3.87, 2.66, and 2.11 min for fettuccini at 160, 170 and 180°F, respectively. At 180°F a >5-log reduction was observed after only 7 min of cooking in both farfalle and fettuccini pasta, far below the recommended labelled cooking instructions.

Significance: The results of this study demonstrate that compliance with labelled cooking instructions will result in an acceptable thermal inactivation of *Salmonella* spp. on dry pasta. The techniques and methodologies used in this study can provide a scientific basis for food safety plans in the dry pasta industry.

P2-171 Validation of Baking of White Chocolate Chip Macadamia Nut Cookie Dough

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Introduction: In 2009, flour was implicated in the *E. coli* O157:H7 outbreak in frozen cookie dough. More recently, macadamia nuts have been recalled due to *Salmonella* contamination. White chocolate chip macadamia nut (WCCMN) cookie dough, containing relatively large or dense chips and macadamia nut pieces, may pose a challenge from a thermal processing standpoint should an ingredient be contaminated. While baked products are generally perceived as microbiologically safe, a FSMA-compliant Food Safety Plan should include data showing that baking yields an appropriate log reduction.

Purpose: The purpose was to determine the log reduction of three specific pathogens in inoculated WCCMN cookie dough after baking.

Methods: Dough samples were individually inoculated with strains of *Salmonella*, *Listeria monocytogenes*, and *E. coli* O157:H7 to achieve target levels of 10^6 to 10^7 CFU/g. Following inoculation, samples were baked at 350°F for 7.5 minutes. After baking, each cookie was analyzed to determine the log reduction for each pathogen.

Results: In WCCMN cookies inoculated with *Salmonella*, *Listeria monocytogenes*, and *E. coli* O157:H7 a minimum 4-log reduction was achieved. For each pathogen category, two trials were conducted on separate days. Each trial consisted of three baking replications of two test cookies for twelve results for each pathogen.

Significance: These data suggest WCCMN cookies (of a specified size) are not a microbiological food safety risk when baked for 7.5 minutes in a 350°F oven. Generally, the FDA uses a minimum 5-log reduction for food safety. However, an internal risk assessment on macadamia nuts determined 4 logs to be adequate, and prevalence data for flour show that contamination levels for pathogens is very low. Purchasing pre-treated nuts or flour is not necessary to ensure food safety as long as these minimum baking parameters are achieved.

P2-172 Validation of Muffin Baking Process to Control *Salmonella* and Determination of Thermal Inactivation Parameters of *Salmonella* in Muffin Batter

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Introduction: The potential presence and stability of salmonellae in low moisture ingredients, such as flour and milk powders, provide a level of risk in further-processed foods. With new FSMA-related regulatory standards for human food, the validation of baking processes to eliminate salmonellae contamination of ready-to-eat bakery products is required.

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Purpose: This study validated a typical commercial muffin baking process as an effective kill-step using a *Salmonella* cocktail of Newport (ATCC 6962), Senftenberg 775W (ATCC 43845) and Typhimurium (ATCC 14028). *D*- and *z*-values in muffin batter were also determined to address variances of baking process parameters.

Methods: Muffins were prepared using inoculated flour (~7 log CFU/g), baked at 190.6°C (oven temperature) for 21 min (mimicking industrial processes), and analyzed by plating samples on selective and injury-recovery media at regular time intervals to determine *Salmonella* reductions. "Breakpoint" time (when no salmonellae were recovered after enrichment) was determined using an extended baking process. *D*-values of the salmonellae cocktail in muffin batter were determined using thermal-death-time disks. Randomized complete block designs were used for baking and *D*-value studies, with three replications as blocks using a = 0.05.

Results: After 21 min of baking, salmonellae counts in the muffins decreased by 6.1 log CFU/g; however, a 5-log reduction was achieved by 17 min. The breakpoint of salmonellae in muffins was 27 min. *D*-values of the *Salmonella* cocktail in muffin batter were 62.2, 40.1 and 16.5 min at 55, 58 and 61°C, respectively; the *z*-value was 10.4°C.

Significance: Baking standard muffins at an oven temperature of 190.6°C for 21 min ensures a >5-log reduction of salmonellae that might be present from contaminated raw ingredients. Presence of inclusions such as fruits, nuts and/or chocolate might impact lethality determinations, prompting additional research. The *D*- and *z*-values determined in this study will help processors design appropriate baking parameters to control salmonellae if pre-baking contamination occurs.

P2-173 Effect of Environmental Stresses on the Expression Levels of Virulence-associated Genes in Shiga Toxin-producing *Escherichia coli*

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) causes severe foodborne illnesses that may result in hemolytic uremic syndrome and death. While *E. coli* O157:H7 remains the main disease-causing serotype, non-O157 STEC has increasingly been associated with illnesses in recent years. Sub-lethal stresses which bacteria experience during food processing may enable the bacteria to survive the subsequent processing and up-regulate the virulence-associated genes.

Purpose: The objective of this study was to establish a relationship between the exposure of O157 and non-O157 STEC to environmental stresses and the expression of virulence-associated genes.

Methods: Four O157:H7 and six non-O157 STEC (O26:H11, O103:H1, O104:H4, O111:NM, O121:NM, and O145:NM strains) strains were repeatedly exposed to sub-lethal chlorine (2 ppm), osmotic (a_w 0.97), or acid (pH 5) stress, followed by quantification of the virulence-associated *stx1*, *stx2*, and *eae* genes using quantitative real-time RT-PCR assay.

Results: When the stressed strains were compared with non-stressed control strains, only chlorine-stressed O103:H1, O104:H4, O145:NM, and O157:H7 showed a significant (P<0.05) increase (>1.4 fold) in relative gene expression of *stx1*. A significant increase in relative gene expression of *stx2* was observed for chlorine-stressed O103:H1 (>2.0 fold) or O26:H11 (1.92 fold) cells, while a slight increase (1.27 to 1.38 fold) in gene expression was observed for O104:H4 and O145:NM cells. Chlorine-stressed cells of O111:NM, O121:NM, and O157:H7 showed similar or a slightly less expression of the selected genes than the control. The relative gene expression levels of *eae* were similar to the patterns of *stx1* and *stx2*, except for stressed cells of O111:NM with a 2.0-fold increase in *eae* gene expression.

Significance: Result showed that STEC had increased expression levels of virulence genes after surviving chlorine stress, suggesting that the increased expression may lead to higher virulence of STEC.

P2-174 Comparative Genome Analysis Reveals a Hyper-virulent *Escherichia coli* O157:H7 Strain Isolated from a Super-shedder

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is a foodborne pathogen that threatens public health on a global scale. STEC O157 predominantly colonizes the terminal recto-anal junction (RAJ) of cattle, which is the major asymptomatic reservoir of this pathogen. Cattle shedding O157 ≥ 10^4 CFU/g of feces are known as super-shedders and are responsible for within-farm and between-farm transmission of STEC O157.

Purpose: The purpose of this study was to perform genetic characterization of KCJ1266, a strain isolated from a super-shedder steer from a farm in North Florida.

Methods: PacBio sequencing was employed for whole genome sequencing (WGS) to characterize the genomic features of KCJ1266. A comparative genome analysis of KCJ1266 with reference genomes including SS17 (strain isolated from a super-shedder), EC4115 (strain related to spinach outbreak) and EDL933 (strain related to hamburger outbreak) was conducted.

Results: WGS of KCJ1266 revealed that it has a genome of 5,478,683 bp encoding 5,545 open reading frames and a plasmid, pO157, of 95,910 bp. *In silico* analysis revealed that KCJ1266 belongs to *E. coli* Lineage I/ II and clade 8, which are related to other disease-causing isolates. In addition, Mauve alignment showed that KCJ1266 shares a similar genomic architecture with SS17 and EC4115. Comparative analyses also revealed that KCJ1266 has the same virulence and similar functional genes as SS17 and EC4115. Phylogenetic analysis showed that KCJ1266, SS17 and EC4115 clustered in the same group. Taken together, these results reveal that super-shedding STEC strain KCJ1266 is a hyper-virulent strain similar to that of SS17 and EC4115.

Significance: This is one of the first studies which utilizes PacBio WGS to characterize a hyper-virulent STEC O157 strain isolated from super-shedding cattle. KCJ1266 can potentially be used as a reference strain for future studies regarding the phenomenon of super-shedding.

P2-175 Prevalence, Isolation, and Genetic Characterization of *Toxoplasma gondii* in Chicken from the United States

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Introduction: *Toxoplasma gondii* is a protozoan parasite that is responsible for approximately 24% of all deaths attributed to foodborne pathogens in the U.S. with an estimated 327 deaths per year, thus making this parasite as a serious food safety concern. Consumption of raw or undercooked meat is the primary infection route for *T. gondii*. Chickens are important vehicles for *T. gondii* and as they are generally fed from the ground, they are used as good indicators for the environmental *T. gondii* oocysts contamination.

Purpose: In this study, we sought to investigate the safety of free-range chickens that are available to purchase by consumers in the U.S.

Methods: Chicken hearts were sampled from the local markets in Maryland and from farms in Maryland and Amish community in Chicago. Each seropositive (modified agglutination test (MAT) positive) heart was bioassayed using Swiss Webster (SW) mice and examined for *T. gondii* infection. In addition, thirteen cryopreserved isolations from previous studies were revived and ten PCR-RFLP markers were used to genotype those isolates.

Results: One hundred fifty from a total of 997 samples (15.0%) were found seropositive for *T. gondii*. No viable *T. gondii* was isolated from chicken hearts that were sampled. All seropositive (150) samples were from a total of 912 samples purchased from local markets.

***Significance*:** The results suggest that *T. gondii* oocysts could present in the environment and infect the food animals. *T. gondii* prevalence in chicken hearts could reflect the environmental contamination of *T. gondii* and prevalence information can be used to manage *T. gondii* infection risk.

P2-176 Genome Sequences of *E. coli* O157:H7 Isolated from 1980s to 1990s

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❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli* of serotype O157:H7 was first recognized in 1982 as a human pathogen associated with outbreaks of bloody diarrhea in the United States and is now considered a major cause of foodborne infections. *E. coli* O157:H7 has caused more outbreaks and hemolytic-uremic syndrome (HUS) cases in the United States than any other serotype. The first major historic outbreak of *E. coli* O157:H7 in the United States occurred in 1993, before the invention of next-genome sequencing.

Purpose: To provide a historical reference for comparison for newly isolated EHEC strains, and to look at the genetics of O157:H7 in the time period of the first outbreaks, we sequenced a collection of EHEC isolates from years 1985 to 1993, isolated from food and animal fecal samples. This date range is important because it includes strains after the first identification of O157:H7 and after the first outbreak in 1993.

Methods: Sixty strains were sequenced by using next-generation sequencing (Illumina Miseq). Twelve of these strains were isolated from food, included milk, salami, meat and mayonnaise. Forty-eight of these strains were isolated from livestock fecal samples.

Results: A total of 54 of 60 samples were positive for O157:H7, 6 of 60 samples were not *E. coli* O157:H7, with 2 of these 6 being *E.coli* serotype O78:H10. 10 of the 54 O157:H7 strains isolated from food samples, while 44 of 54 the O157:H7 strains isolated from livestock fecal samples. Forty strains carried *stx1*, 46 strains carried *stx2*, and total of 35 strains carried both *stx* genes that these strains isolated from these dates are highly similar to more recent isolations.

Significance: These new historical reference sequences can be useful references library and valuable resources for further research of *E. coli* O157:H7. These strains have probably been causing disease even before the major outbreaks, but mostly unreported because we do not see evidence for a major evolutionary shift in the years between the recent expansion of whole genome sequencing and this reference library.

P2-177 Applying Next Generation Sequencing to Subtype *Listeria monocytogenes* Isolates from Fish-processing Facilities

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Introduction: Listeriosis is the third leading cause of death from infections caused by foodborne pathogens. Listerial infection occurs through ingestion of foods contaminated with *Listeria monocytogenes*. This pathogen has a wide distribution in different environments and a strong capability to survive under various stressful conditions. Long-term presence of *L. monocytogenes* in food processing environments poses a difficult challenge for public health and food safety.

Purpose: The purpose of this study was to evaluate next generation sequencing technology as an applicable subtyping tool for compliance inspection of *L. monocytogenes* contamination for smoked fish-processing facilities and food recalls.

Methods: Sequencing genomic libraries of *L. monocytogenes* isolates derived from five smoked fish-processing facilities over ten years were prepared using the Illumina Nextera XT kit and subsequently applied for 2×250 bp paired-end sequencing runs on the Illumina MiSeq sequencer. The raw data of fastq files were imported as paired reads into the Qiagen CLC Genomics Workbench for further data analysis and SNP-based phylogenetic tree construction.

Results: We have successfully sequenced 71 *L. monocytogenes* genomes from those fish-processing facilities using the Illumina MiSeq sequencer. By using K-mer based spectra, 7 best matched references derived from NCBI genome database were yielded for those 71 genomes, among which 35% (25/71), 25% (18/71) and 30% (21/71) of *L. monocytogenes* genomes matched to 3 core references. K-mer based phylogenetic analysis revealed NZ_HG813249 as the common reference for further analysis. We were able to yield average coverages of 120±34 for sequencing depth, (95.3±2.4)% of mapped reads and 64,832±56,355 high quality variants (range 198-254,281) when mapping reads to the common reference. SNP-based phylogenetic analysis revealed 14 clades resulting in 3 large ones containing 20, 16 and 18 genomes, respectively.

Significance: Our data indicate that next generation sequencing is a valuable subtyping tool for analyzing *L. monocytogenes* isolates from smoked fish firms.

P2-178 *Listeria monocytogenes actA* Polymorphism Isolated from Food, Carcass, and Human in South Korea

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Introduction: *Listeria monocytogenes* is capable of survival in various environments and highly pathogenic (mortality: 20-30%) foodborne bacteria. ActA is a bacterial membrane protein, which encoded by 268-bp or 385-bp *actA* genes, and it induces intracellular or intercellular spreading by actin filament.

Purpose: The objective of this study was to compare the pathogenicity of *L. monocytogenes* between 268-bp and 385-bp *actA*.

Methods: To confirm the *actA* polymorphism of *L. monocytogenes* isolated from food, carcass, and humans in South Korea, primers were prepared and analyzed with PCR. *actA* sizes were identified by electrophoresis. To compare the invasion efficiency into Caco-2 cells between two *actA* genes, each of the *L. monocytogenes* strains (OD_{625} = 0.01) were infected to monolayer Caco-2 cell [young (2 day old) versus old aged (10 day old)], followed by incubation in 5% CO_2 at 37°C for 24 h. After incubation, the Caco-2 cells were treated with gentamicin and triton X-100, and *L. monocytogenes* cell counts were then enumerated on tryptic soy agar plus 0.6% yeast extract.

Results: The size of most domestic food and carcass isolates had 385-bp *actA*, but the size of most human isolates had 268-bp *actA*. In addition, *L. monocytogenes* strains with 385-bp *actA* showed obviously high Caco-2 cell invasion efficiency, compared to *L. monocytogenes* strains with 268-bp *actA* ($P<0.05$). However, the invasion efficiency of *L. monocytogenes* strains with 268-bp *actA* became similar to those of *L. monocytogenes* strains with 385-bp *actA* in old aged Caco-2 cell, compared to young aged Caco-2 cell.

Significance: These results suggest that the size of *actA* may be related to *L. monocytogenes* invasion efficiency.

P2-179 Comparing Growth Kinetics of *Listeria* spp. Isolates from Pastured Poultry to Varied Sources

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Introduction: Pastured poultry is an alternative rearing method that is becoming more popular because many consumers perceive its benefits of nutrition, safety, and welfare. However, greater access to the outdoors leads to exposure to saprophytic, soil-borne organisms such as *Listeria* spp., with very little known regarding the growth patterns of these isolates.

Purpose: This study compared the growth rates of *Listeria* spp. isolates from pastured poultry to other isolates from conventional poultry, humans, and plant matter.

Methods: Maximum specific growth rates were calculated from growth curves obtained using a micro plate reader, tube optical density (OD) readings, and plate counts (CFU/ml).

Results: Quadruplicate microplate reader experiments revealed that the ten pastured poultry strains had overall higher maximum growth rates ($P<0.05$) than the ten isolates from varied sources. *L. innocua* F6-840 and *L. welshimeri* F6-844, pastured poultry isolates, and *L. monocytogenes* EGD-e, a non-pastured poultry isolate, were then selected for growth measurements via plate counts and tube OD readings. There were differences in growth rates ($P<0.05$) between plate counts and tube OD readings compared to microplate reader data for *L. innocua* F6-840 and *L. welshimeri* F6-844. In all three strains, maximum growth rates from microplate reader data were less ($P<0.05$) than plate counts and tube OD readings. *L. innocua* F6-840 had higher growth rates ($P<0.05$) than *L. welshimeri* F6-844 and *L. monocytogenes* EGD-e, but this was observed only with microplate reader data. Plate count maximum growth rate from triplicate experiments for *L. innocua* F6-840 was 0.964 (±0.03), for *L. welshimeri* F6-844 the rate was 0.855 (± 0.12), and for *L. monocytogenes* EGD-e was 1.287 (± 0.31).

Significance: Understanding the physiological characteristics, such as maximum growth rates, of *Listeria* strains isolated from pastured poultry aids in developing more accurate risk assessments for this rearing method and furthers our understanding of their ecology.

P2-180 Comparison of Thermal and Pressure-assisted Thermal *D*-Values of Non-proteolytic *Clostridium botulinum* Types B and F

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Introduction: The impact of high pressure processing (HPP) on the inactivation of nonproteolytic spores of *Clostridium botulinum* is important for extended shelf life refrigerated low-acid foods.

Purpose: Study and compare the thermal and pressure-assisted thermal resistances of the most resistant nonproteolytic *C. botulinum* strains as selected from our previous screening study of 17 strains.

Methods: Spores of nonproteolytic *C. botulinum* strains, Ham-B, Kap 9-B and 610-F were prepared using biphasic media and diluted in ACES buffer (0.05M, pH 7) to 10^5-10^6 CFU/ml and placed into a modified sterile transfer pipette, heat-sealed and subjected to a combination of temperatures (80-91°C) and high pressures (600-750 MPa) in a laboratory and pilot-scale high pressure test systems. Another set of diluted spores were placed in NMR tubes which were heat-sealed at both ends, and subjected to various temperatures (80-91°C) in a Fluke 7321 High Precision Bath with Duratherm S as heat transfer fluid. Survivors were determined by 5-tube MPN method using TPGY broth after incubation for 3 months at ambient temperature.

Results: Both thermal and pressure-assisted thermal *D*-values (min) of Ham-B, Kap 9-B, and 610-F decreased as the process temperature increased. The highest log reduction of spores (> 5.0) occurred at 91°C and with the high temperature and pressure combination (91°C and 750 MPa). Pressure-assisted thermal *D*-values were consistently higher for Ham-B, Kap 9-B, and 610-F when processed at any temperature/pressure combination when compared to those processed at any temperature without application of pressure. Thermal *D*-values at 87°C for Ham-B, Kap 9-B and 610-F were 0.48, 0.53, and 0.37, respectively, compared to those at the 87°C combined with 600 MPa which were higher (i.e., 3.52 for Ham-B, 3.33 for Kap 9-B, and 5.34 for 610-F).

Significance: Overall, pressure-assisted thermal *D*-values of Ham-B, Kap 9-B, and 610-F were higher compared to their thermal *D*-values at the same temperature. It appears that within the range of pressures evaluated, combination of high pressure with temperature has a protective effect on spores of *C. botulinum* nonproteolytic types B and F strains based on the thermal and pressure-assisted *D*-values.

P2-181 Heat Inactivation Kinetics of *Staphylococcus carnosus* Chr CS-299 a Potential Surrogate for Hepatitis A Virus

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❖ ***Developing Scientist Competitor***

Introduction: Thermal treatment is a common method for inactivating microbial pathogens in a wide range of food products. Recent studies have shown that hepatitis A virus (HAV) has a $D_{72°C}$ of 0.9 min in buffer which is greater than vegetative bacterial pathogens. Common surrogates, such as *Listeria innocua*, are not resistant enough to be used as surrogates for HAV and thus, new surrogates need to be identified.

Purpose: The purpose of this study was to compare the thermal inactivation kinetics (*D*- and *z*-values) of *Staphylococcus carnosus* at different incubation temperatures to identify a potential surrogate for HAV.

Methods: Thermal inactivation of *S. carnosus* Chr CS-299 was performed in phosphate buffered saline (PBS) following incubation at 32°C or 48°C. A total of 7.6 log CFU/ml of *S. carnosus* in PBS was added to 2 ml vials. Thermal inactivation studies were performed at 65, 67, and 70°C. Vials were removed at various time points, cooled in an ice bath, plated on BHI agar and incubated for 72 h at 32°C or 48°C. Each trial was conducted in duplicate and replicated three times. *D*- and *z*-values were determined using a first-order model.

Results: For 32°C incubation, *D*-values for *S. carnosus* in PBS were 1.59 ± 0.2, 0.91 ± 0.1 and 0.34 ± 0.05 min, at 65, 67, and 70°C, respectively, with a *z*-value of 7.46°C. For 48°C incubation, *D*-values were 3.4 ± 0.16, 1.43 ± 0.01 and 0.48 ± 0.03 min, at the same respective temperatures, with a *z*-value of 5.93°C. Thus, using 48°C incubation, *S. carnosus* could be a potential surrogate for HAV ($D_{72°C}$= 0.9 min).

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Significance: Based on inactivation kinetics, ease of incubation, and non-pathogenicity, *S. carnosus* could be used for validation studies of HAV. Additionally, it was shown that a significant increase in heat resistance could be achieved by increasing the incubation temperature.

P2-182 Heat Resistance of *Salmonella* Tennessee when Heat Treated in Liquid Medium

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Introduction: *Salmonella enterica* is one of the most common causes of foodborne illness in the USA. Although infrequently reported from food source, *Salmonella* serovar Tennessee was associated with a peanut butter outbreak in 2007, which highlights the risk of salmonellosis from heat-processed foods.

Purpose: The objective of this study was to investigate the heat resistance of *Salmonella* Tennessee when heat-treated in liquid medium.

Methods: Twelve isolates, including *Salmonella* Tennessee, Kentucky, Cubana, and Senftenberg were included. *Salmonella* Senftenberg 775w was used as positive control. Overnight cultures were heat treated at 54, 60, or 70°C for 20 min in a water bath. A subset of cells was inoculated into LB broth after the water activity had been adjusted to 0.97. These cultures were grown overnight and heat-treated as above. Afterwards the cells were recovered and counted. Plotting the log of cell survivors against time allowed for calculation of *D*-values.

Results: *Salmonella* Senftenberg 775w demonstrated a $D_{54°C}$ value of 71.9 min and $D_{60°C}$ value of 7.2 min, which is within the range of and consistent with previous published reports for this reference strain. *Salmonella* Tennessee CFSAN001345, isolated from peanut butter during a food contamination event in 2007, showed a $D_{54°C}$ value of 21.1 min, which was 1.5 fold greater compared to other *Salmonella* Tennessee, Kentucky and Cubana isolates that had a $D_{54°C}$ value ranging from 8.8 to 14.5 min. Strain and serovar variations were observed, which agreed with studies published previously.

Significance: Future transcriptional studies should provide more information on the epigenetic forces that drive thermal resistance in this strain and studies in foods may point, in part, to mechanisms underlying *Salmonella* Tennessee's ability to adapt and survive in peanut butter and its production environment. Moreover, understanding heat resistance in *Salmonella enterica* can aid in evaluating efficacy of *Salmonella* control measures after heat-treatment of processed foods.

P2-183 Determination of Thermal Inactivation Parameters and Lethality of *Salmonella* spp. during Whole-Grain Bread Baking

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Introduction: Raw baking ingredients, such as flour, can become contaminated with *Salmonella* spp., which can survive for extended periods in these low-moisture matrices. Contaminated flour has been identified as a source of salmonellosis in humans. Although bread is subjected to thermal treatment during production, published literature is lacking for validation of the baking process as a kill-step for *Salmonella* spp.

Purpose: To determine the thermal inactivation parameters (*D*- and *z*-values) for *Salmonella* spp. in whole-grain bread dough, and to validate a representative commercial baking process as an effective pathogen kill-step during bread production.

Methods: Whole-grain flour was inoculated with a 7-strain cocktail of *Salmonella* spp. [four food production facility isolates, and three ATCC strains (Senftenberg 775W 43845, Newport 6962, and Typhimurium 14028], formed into dough, and either baked at 190.6°C for 35 minutes simulating a commercial baking process or transferred to thermal death time disks and heated in hot water baths at 50, 52, or 55°C for 30 to 80 minutes. Surviving *Salmonella* spp. populations were determined by plating on injury recovery and selective agars. Three replications were conducted with randomized complete block design. *D*- and *z*-values were determined by linear regression.

Results: *Salmonella* spp. populations were undetectable after enrichment (>6 log CFU/g reductions) following 16 minutes of baking. *D*-values of the 7-strain *Salmonella* spp. cocktail in bread dough were 59.6, 20.0, and 9.7 at 50, 52, and 55°C, respectively. The *z*-value of *Salmonella* spp. was 6.5°C. No significant differences ($P > 0.05$) in *Salmonella* spp. recovery were observed between the injury recovery and *selective* agars.

Significance: Baking at 190.6°C reduces *Salmonella* spp. populations in bread by >6 log CFU/g to non-detectable populations, thus providing an effective kill-step for *Salmonella* spp. during typical commercial baking processes. Established *D*- and *z*-values in whole-grain bread dough will assist bakers in process lethality determinations and modeling.

P2-184 Microbial Evaluation of Pre- and Post-processed Tomatoes from Florida Packinghouses

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Introduction: Prevention of microbial cross-contamination during postharvest handling is an important step to minimize the microbial food safety hazards. Dump tanks (i.e., flume systems) are widely used in states like Florida to transfer/wash tomatoes, and are one of the most critical points where cross-contamination can be prevented.

Purpose: The main objective of this study conducted during the 2013 and 2014 harvesting seasons at five growing regions in Florida was to evaluate the efficiency of post-harvest processing of tomatoes in commercial packinghouses.

Methods: Determination of total aerobic plate count (APC), total coliforms (TC) and generic *E. coli* (EC) on 720 composite samples (five tomatoes per sample) both before and after processing were carried out to accomplish this objective. APC was determined using standard plate count agar (Thermo Fisher Scientific, Waltham, MA) and TC and EC were determined using ECC CHROMagar (DRG International, Inc., Mountainside, NJ). One hundred ml of 0.1% (w/v) sterile peptone water (PW) (Thermo Fisher Scientific, Waltham, MA) was added to the sterile sample bags with tomatoes and rubbed for 60 s. One hundred µl of each dilution was spread plated onto appropriate agar plates.

Results: The least square mean (LSM) value of APC for pre-processed samples was 6.6 log CFU/tomato, which was significantly lower ($P<0.0001$) in post-processed tomatoes to 5.8 log CFU/tomato. Similarly, the LSM for TC counts was 4.4 log CFU/tomato in pre-processed samples, whereas it reduced to in 4.1 log CFU/tomato in post-processed samples. Eighty-six out of 720 (11.9%) and 701 out of 720 (97.3%) 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 generic *E. coli* in pre- and post-processed samples. The APC and TC counts showed significant ($P<0.0001$) seasonal variation.

Significance: Information from this study is suitable for determining areas in which improvements may be made to optimize standard post-harvest operational procedures to ensure food safety and subsequently control disease outbreaks.

Poster

P2-185 *Listeria monocytogenes* Survival and Growth in Milkshakes Made from Artificially- and Naturally-contaminated Ice Cream

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❖ ***Developing Scientist Competitor***

Introduction: A foodborne listeriosis outbreak spanning from 2010-2014 was linked to consumption of ice cream contaminated with *Listeria monocytogenes*. The outbreak resulted in 10 listeriosis cases, leading to 100% hospitalizations and 33% mortality rate. The deaths were attributed to consumption of milkshakes made with pre-portioned ice cream scoops in a healthcare setting. Work by other FDA investigators during the outbreak demonstrated uniform contamination of the scoops at approximately 10 MPN/g.

Purpose: To evaluate the survival and growth of *L. monocytogenes* in milkshakes prepared with artificially- and naturally-contaminated scoops.

Methods: For each trial, half of the scoops were inoculated with 10 CFU/g of a rifampicin-resistant *L. monocytogenes* 4b cocktail (F2365, LS806, R2-502, and ScottA) directly with a pipet tip inserted into the sample. The other half of the scoops remained as naturally-contaminated with multiple *L. monocytogenes* 4b strains. Milkshakes were prepared according to the outbreak-associated recipe (1 scoop blended with 2 oz 1% milk). For this study, UHT milk was used and milkshakes were stored at 25°C for 0, 6, 9, 12, 24, and 48 h, followed by enumeration of *L. monocytogenes* via MPN according to the FDA-BAM. Twenty-four and 48 h enrichments were streaked onto PCA^{Rif} or Brilliance *Listeria* agar for detection.

Results: The artificially-contaminated *L. monocytogenes* achieved significantly higher (P<0.05) populations than the naturally-contaminated at both 12 and 48 h. At 12 h, the populations of naturally- and artificially-inoculated *L. monocytogenes* were 1.26±0.45 and 2.22±0.29 log MPN/g, respectively. At 24 h, both populations were approximately 4.25 log CFU/g and not significantly different. At 48 h, populations increased to 4.82±0.18 and 6.65±0.11 log CFU/g, respectively.

Significance: This study highlights the difference in survival between an artificial and natural contamination of *L. monocytogenes*. These results may help to inform risk assessment, which often depends on studies using artificial contamination.

P2-186 Microbiological Growth Profile of *Staphylococcus aureus* in Pretzel Bread Dough Systems during Routine Manufacturing Conditions

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Introduction: Products with a_w > 0.91 and a pH > 4.5 and < 9.6 may permit growth of *Staphylococcus aureus* and, therefore, potential heat stable enterotoxin formation. Dough systems (a_w > 0.91) used for pretzels and pretzel products may have the potential to support the growth of *S. aureus* during routine production.

Purpose: The purpose of this study was to evaluate the microbiological profile of dough systems during routine production conditions from a food safety standpoint.

Methods: Dough samples were collected from a commercial pretzel facility. Two types of dough: (1) regular pretzel dough and (2) peanut butter containing pretzel dough were evaluated for the study. Dough samples were individually inoculated with multiple strains of *S. aureus* to achieve a target level of 10^2 CFU/g. Following inoculation, samples were stored at 25 and 35°C. Samples were analyzed following storage at several time points between 0 and 7 days. Baird-Parker agar, deMan-Rogosa-Sharpe agar, Potato Dextrose agar supplemented with Chlortetracycline and Tryptic Soy agar were used as plating media for *S. aureus*, Lactic acid bacteria, Yeasts and Total Plate counts, respectively. The experiment was performed in triplicate. In accordance with FDA published guidance, the limit of food safety was defined as 10^5 CFU/g for *S. aureus*. ANOVA analysis was performed to understand the effect of storage temperature and time on the growth profile of *S. aureus*. The level of significance used was 5%.

Results: Growth of *S. aureus* did not exceed (P<0.05) the limit of food safety at both temperatures in all the evaluated dough types. This may be attributed to 'competitive inhibition' due to the presence native flour microflora.

Significance: The study findings indicate no significant food safety risk associated with the current production practices. The data generated in this study also provide scientific basis for the facility's food safety plan in compliance with anticipated FSMA guidelines.

P2-187 Microbiological Contamination Analysis in Kimchi and the Ingredients for Food Safety

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Introduction: Kimchi is the best-known Korean traditional fermented food and is made through the fermentation of vegetables. Microbiological contamination analysis in Kimchi is very important because of spontaneous fermentation without the sterilization of raw materials in Kimchi preparations.

Purpose: This study was performed to evaluate the microbial contamination levels of 100 commercial Kimchi samples and 200 ingredients samples such as dried red pepper powder, ginger, radish, garlic and onion. In addition, washing treatment was examined for its effectiveness in reducing microbial contamination levels in minor ingredients of Kimchi.

Methods: Populations of sanitary indication bacteria (total aerobic bacteria, coliforms, *Escherichia coli*) and pathogenic bacteria (*Bacillus cereus, Enterohemorrhagic E. coli*, *Clostridium perfringens*, *Campylobacter jejuni/coli*, *Staphylococcus aureus*, *Salmonella* spp., *Vibrio parahaemolyticus*, *Listeria monocytogenes*, and *Yersinia enterocolitica*) were investigated in all samples.

Results: In case of commercial Kimchi, total aerobic bacteria and coliforms were detected at the levels of 4.7~8.9 log CFU/g and 0~3.9 log CFU/g, respectively. Among minor ingredients, microbial contamination in ginger showed particularly higher than others. Levels of total aerobic bacteria and coliforms in ginger samples were 5.3~8.8 log CFU/g and 2.8~7.3 log CFU/g, respectively. For pathogenic bacteria, *B. cereus* ranged between 0~4.0 log CFU/g in all samples while others were not detected. The optimized washing treatment led to a decrease in the number of sanitary indication bacteria and pathogenic bacteria in all samples. For ginger samples, the reduction values of total aerobic bacteria and coliforms after washing treatment were 0.2~3.2 log CFU/g and 0.3~2.7 log CFU/g, respectively. Among the pathogenic bacteria, reduction values of *B. cereus* were 1.0~3.9 log CFU/g.

Significance: According to these results, the reduction of microbial contamination in its ingredients is key-point to ensure the safety of Kimchi. Moreover, these results indicated that appropriate washing strategy could reduce microbial contamination level of commercial kimchi. Thus, there is an urgent need for a washing treatment system to control the microbiological risk factors.

P2-188 Molecular Subtyping of *Clostridium botulinum* Isolates Associated with an International Outbreak of Foodborne Botulism from Commercial Carrot Juice

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Introduction: In 2006, commercial carrot juice was the source of a foodborne botulism outbreak involving both Canada and the United States. *C. botulinum* strains isolated from clinical specimens and food split into two major botulinum neurotoxin (BoNT) subtypes: A1 and A(B). The BoNT/A1 strains contained a unique gene cluster (ha-/orfx+) rarely associated with BoNT/A1 producing strains.

Purpose: Evaluate genetic variation in outbreak-associated *C. botulinum* ha-/orfx+ subtype A1 isolates using whole genome sequencing.

Methods: Six *C. botulinum* ha-/orfx+ subtype A1 outbreak-associated isolates from stool (CDC51291, CDC51297, CDC51289), implicated food (CDC51303), unopened bottles (CJ4-1, CJ5-1), and an ha-/orfx+ subtype A1 strain (CDC1882) unrelated to the outbreak were examined. Sequencing was performed on an Illumina MiSeq or Ion Torrent PGM. BoNT genes were compared using CLC Genomics Workbench. SNP analysis, using reference strain CDC297, was performed using RealPhy v.112, and SNP phylogenies constructed with MEGA6. Gene annotations were performed with the Rapid Annotation using Subsystem Technology (RAST) online tool.

Results: Most outbreak-associated isolates were similar and differed from the reference genome of CDC297 by 20 to 60 SNPs; however, CJ5-1 differed by 152 SNPs. Strain CJ5-1 contained 2 unique phage associated genes and several open reading frames not detected in the other isolates. CJ4-1 and CJ5-1 contained phage introns and genes encoding DNA replication proteins not present in CDC297 or in the other outbreak related isolates. The *bont/A1*gene of CJ5-1 was identical to that of strain CDC1882 and shared 1 SNP difference to CDC297 and the other outbreak related isolates.

Significance: Whole genome SNP analysis provided a rapid, robust, and high resolution method for subtyping isolates from a foodborne botulism outbreak. Data from whole genome sequencing (WGS) was used to identify bacteriophages and other mobile genetic elements which can be used to better understand the phylogenetic diversity of botulinum neurotoxin-producing clostridia and aid in botulism outbreak detection.

P2-189 Seasonal Effect on Diversity and Dynamics of Microbiota during Preparation and Ripening of Chihuahua Cheese Made from Unpasteurized Milk

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Introduction: Chihuahua cheese is a product usually elaborated from cow´s raw milk by diverse and traditional process. Yearly variations on chemical and microbiological composition of milk, as well as non-standardized manufacturing process have been identified as the possible cause of variations of cheese characteristics and microbial safety.

Purpose: To evaluate seasonal influence on microbial diversity and dynamic during elaboration and ripening of traditional Chihuahua cheese.

Methods: Five cheese factories (A-D) were visited three times during a year in different seasons and portions of raw milk, curd (after cutting), cheddaring (before salting) and freshly prepare cheese were obtained; also, portions of cheese ripened in the laboratory (30-270 d) were taken and conserved at -20°C until analysis. From a total of 144 samples, genomic DNA was extracted and the V3 region of the*16S rRNA* gene was amplified; amplicons were separated using denaturing gradient gel electrophoresis (DGGE) by a linear denaturing gradient of 30-70% of urea and formamide.

Results: The banding pattern of manufacturing samples exhibited between 4 and 18 different bands, noticing more microbial diversity on farm A (18 bands); ripening cheese samples showed 5 to 13 different bands, being cheeses form factory E the highest (13 bands). Some bands prevailed during all the period of study, other appeared during ripening, and a few disappeared or the band was less intense throughout the manufacturing and ripening processes. Other bands could only be observed in a dairy or in a season in particular, which is a clear indication of the effect of season and the particular manufacturing practices and conditions of each dairy on the dynamic and diversity of microorganisms.

Significance: It was possible to describe the dynamics and diversity of microorganisms involved on the manufacturing and ripening of a traditional cheese made from raw milk, using DGGE as analytical tool. Seasonal and location variations were observed.

P2-190 Seasonal Influence on Microbial Diversity of Chihuahua Cheese Elaborated from Raw Milk

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Introduction: Artisanal Chihuahua cheese is principally elaborated by the Mennonite Community in Northern Mexico, using raw cow's milk for its artisanal elaboration. It is characterized by its intense flavor and aroma, but some variations in these attributes can be distinguished as a consequence of seasonality.

Purpose: To evaluate seasonal influence on microbial isolates from artisanal Chihuahua cheese elaborated with raw milk.

Methods: Samples of raw milk, curd, cheddaring and cheese were collected during three seasons of the year from a Mennonite farm. Cheese was aged on the laboratory, and a portion was taken every 30 d during 270 d. From a total of 11 types of samples, decimal dilutions were prepared to spread 100 µl on MRS and Elliker agar and incubated at 25 and 42°C. Isolated gram-positive colonies were selected to DNA extraction and amplification of 16S *rRNA* gene. RFLP analysis was made by the restriction enzyme MboI.

Results: A total of 181 bacterial isolates were analyzed, and the highest numbers were isolated from cheese samples. There was a prevalence of cocci strains (151), and only 30 bacilli strains. Analysis of *16S rRNA* gene by RFPL allows the identification of 11 different groups with different band pattern. Bacterial strains isolated during autumn were located on categories 1, 2, 4 (all bacilli) and 10 and 11 (both cocci); while winter isolates were located in groups 3 (cocci) and 8 (bacilli); summer strains belong to group 7 (cocci). Groups 5, 6 and 9 (all cocci) are formed by strains isolated during the three seasons, which might be strains typical of the dairy environment.

Significance: Analysis of the microbial isolates of native Chihuahua cheese by *16S rRNA* gene shows the marked influence of the season on strain distribution, but there are also strains that prevail on the dairy samples independently of the season.

P2-191 Modulation of the Gut Microbiota by Tart Cherries Consumption: In vitro and Human Dietary Intervention Studies

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Poster

Introduction: Dietary polyphenols have long been considered beneficial, but these health claims have mainly been based on the properties of the native polyphenols. However, the vast majority of plant/fruit polyphenols molecules are too big to be directly absorbed in the small intestine. It is becoming increasingly evident that dietary polyphenols are converted to smaller metabolites by the resident microbiota, mainly in the colon. Therefore, understanding both how the gut microbiota is affected by polyphenol consumption and the fate of said polyphenols is crucial to solidify health claims. Tart cherries have been shown to exert health benefits, and this has been attributed to their specific and abundant polyphenolic content.

Purpose: The aim of this study was to conduct preliminary studies in vitro and in vivo to assess the potential prebiotic effect of concentrate tart cherries juice.

Methods: Tart cherry juice concentrates, as well as representative polyphenol extracts, was used to set up in vitro assays (fecal slurries, simulator of the human intestinal tract microbial ecology) from which fecal samples were obtained and subjected to 16S rRNA gene sequence high throughput sequencing (MiSeq) and metabolomics through HPLC-qTOF-MS. A short-term dietary intervention study was conducted with 10 participants consuming the recommended amount of tart cherry juice daily over five days. Fecal samples were taken before and after the dietary intervention and subjected 16S rRNA gene sequence high throughput sequencing

Results: In the in vitro study, both tart cherry juices and chlorogenic acid (the main polyphenolic constituent of the juices) resulted in a significant increase of *Lactobacillus* and Bifidobacterium, correlating with an increase in *Ruminococcus*. The metabolomics indicated that, in contrast with sweet cherry juice, tart cherries polyphenols where predominantly converted to coumaric acid. A very recent indepedent study supported those microbiota and metabolomics trends at least for chlorogenic acid. Importantly, similar trends in gut microbiota were observed in most human subjects. It appeared that low initial abundance of *Ruminococcus* is limiting the "prebiotic" impact of tart cherries, potentially making it a keystone species.

Significance: To conclude, our results indicate a potential "prebiotic" effect of tart cherries polyphenols on the human healthy gut microbiota. Additional studies are under way to determine the metabolomics trends in the human samples, as well as metagenomic studies. Future studies are envisioned that would include strategies to stimulate Ruminococcus, thereby maximizing the prebiotic effect.

P2-192 Investigation of Erythromycin-resistant *Campylobacter jejuni* from Turkey Farms in North Carolina

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Introduction*: Campylobacter* is a leading cause of bacterial foodborne illness and is often linked to contaminated poultry. Macrolides such as erythromycin are the drugs of choice when treatment is indicated. Even though erythromycin resistance is frequent among *Campylobacter coli*, it remains rare in *C. jejuni* and was not encountered during several years of surveillance of *Campylobacter* from turkeys in North Carolina. However, from 2014-2016, three North Carolina turkey flocks were found to be colonized by erythromycin-resistant (ErmR) *C. jejuni* strains.

Purpose: The purpose of this study was to determine the prevalence of ErmR *C. jejuni* in turkey farms and identify the genetic cause of the resistance.

Methods: *Campylobacter spp.* from turkey cecal or fecal samples and from flies in turkey farms were isolated on selective media (mCCDA), speciated via multiplex PCR, characterized for antibiotic resistance and genotyped by multilocus sequence typing (MLST). A 23S rRNA gene fragment was amplified and sequenced to identify mutations known to associate with macrolide resistance.

Results: ErmR *C. jejuni* was identified in 0.53%, 0.91%, and 3.7% (since 3/2/16) of the flocks from 2015-2016, respectively. Of the three flocks with ErmR *C. jejuni*, one yielded isolates with ST-1839 and another ST-7729, which was novel and differed in all seven alleles from ST-1839. Previously identified ST-1839 isolates were multidrug resistant but were erythromycin sensitive. The ErmR *C. jejuni* isolates harbored the 23S rRNA mutation associated with macrolide resistance.

Significance: Although uncommon in turkey flocks, flocks colonized by ErmR *C. jejuni* were identified. The presence of a macrolide resistance associated mutation in a chromosomal locus suggests the potential for dissemination to other *C. jejuni* via transformation. These data will enhance the currently limited knowledge regarding ErmR *C. jejuni* in food animals and will contribute to further surveillance on the prevalence of ErmR *C. jejuni* in poultry.

P2-193 Prevalence of Resistant *Salmonella* spp. in Drinking Water Sources in Nyankpala Community, Ghana

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Introduction: The contamination of drinking water by *Salmonella* spp. and their resistance to antibiotics is a threat to public health and global concern.

Purpose: This work determined the prevalence of resistance *Salmonella* spp. isolated from drinking water sources in Nyankpala Community of Ghana.

Methods: Two hundred seventy-five drinking water samples were examined. Isolation of *Salmonella* spp. was done according to USA FDA-BAM. Antibiotic susceptibility test was performed for 34 *Salmonella* spp. using the disc diffusion method.

Results: Of the 275 drinking water samples examined 46.9% (12) were positive for *Salmonella* spp. Water samples collected from dam (4 of 5) and well (4 of 25) were the most contaminated, followed by rain water (stored) (3 of 25) and tap water (1 of 35). Bottle (0 of 25), sachet (0 of 100) and water trough (0 of 40) water samples were negative for *Salmonella* spp. There were no significant differences (P>0.05) among water samples that were positive for *Salmonella spp*., however, dam- and well-positive samples differ significantly (P<0.05) from bottle water, sachet water and water trough samples. From the 34 *Salmonella* isolates examined against 9 different antibiotics, 100% and 94.12% were resistant to Erythromycin and Vancomycin, respectively. The 34 *Salmonella* isolates also exhibited six different antibiotic resistant patterns. Twenty-three (23) of the isolates exhibited a resistant pattern of VA-E with MAR index of 0.22, five exhibited a pattern of VA-E-AMC with MAR index of 0.33, two isolates exhibited a pattern of VA-E-CRO with MAR index of 0.33, one exhibited a pattern of VA-E-C with a MAR index of 0.33, two exhibited a pattern of VA-E-CRO-AMC with MAR index of 0.44 and one exhibited a pattern of VA-E-AMC-CN with MAR index of 0.44.

Significance: This study creates the awareness that some drinking water sources in Nyankpala Community of Ghana are contaminated with *Salmonella* spp., which are resistant to some antibiotics especially Erythromycin and Vancomycin. Therefore, consumers of water in this Community are at risk of *Salmonella* infection.

Poster

P2-194 Effect of Adaptation to Acetic Acid and Low pH on the Acid Resistance of *Salmonella enterica* ssp. *enterica serovar* Enteritidis in Laboratory Medium and Mayonnaise

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❖ ***Developing Scientist Competitor***

Introduction: Hurdle technology has been widely used for food safety. However, microorganisms are able to activate mechanisms that help them survive under adverse conditions, thus compromising the effectiveness of hurdles.

Purpose: To investigate the adaptive responses of *Salmonella* Enteritidis to lethal pH (laboratory medium, mayonnaise) induced by exposure to sublethal acid conditions (low pH in the presence of undissociated acetic acid).

Methods: Tryptone Soy Broth without dextrose (TSB Glu(-)) was used for growth and adaptation of *Salmonella*. Acid challenge was performed in TSB adjusted to pH 2.5 with HCl and mayonnaise. For broth experiments, different concentrations of total acetic acid (AA) were used (15, 25 and 35 mM) and the pH was adjusted to 4.0, 4.5, 5.0, 5.5, 6.0 using HCl/NaOH. Non-AA adapted cells (0m M/pH 4.0, 4.5, 5.0, 5.5, 6.0) were also inoculated. Two of these acetic-adaptation cases showing the highest (15 mM/pH 6) and lowest reduction (35 mM/pH 5.5) together with non-AA adapted cells (0 mM/pH 5.5 and 6.0) were selected and used as adaptation inocula to commercial packages of mayonnaise (initial pH 3.9) stored at two temperatures. Non-adapted cells were those grown in non-acidified TSB Glu(-). Experiments were conducted twice in two replicates.

Results: In broth experiments, cells adapted with AA were countable for longer time compared to those pre-exposed to HCl. This was more profound within a range of undissociated AA (e.g. 35 mM/pH 5.5). Non-adapted cells had the fastest reduction. However, in mayonnaise experiments, non-adapted cells together with cells adapted to AA 35 mM/pH 5.5 remained countable for longer time compared to the other inocula. At the end of storage period, *Salmonella* was detectable (enrichment protocol) in all samples.

Significance: Results can provide new information regarding the impact of exposure to sublethal acid conditions on subsequent resistance of *Salmonella* Enteritidis and may help food industries develop safe food formulations.

P2-195 Effect of Growth Media on Bacterial Pressure Resistance of *Escherichia coli* K12 lux Bioreporters

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Introduction: Hydrostatic pressurization has revolutionized traditional food preservation as its application on foodstuffs results in inactivation of undesirable microorganisms. The lack of understanding about the microbial metabolic changes occurring under pressure however has hindered the widespread use of this technology. In addition, pressure induced microbial inactivation seems to be strain and environment dependent.

Purpose: The purpose of this study was to evaluate the effect of nutrient rich and nutrient deficient growth media on pressure resistance of *Escherichia coli* K12 lux.

Methods: *Escherichia coli* K12 lux strains were grown in 100 ml nutrient rich Luria-Bertani broth and nutrient deficient M9 media, incubated for 16 hours at 37°C and sub-cultured in 100 ml fresh LB broth and M9 media until an OD_{600} ~0.5 was reached. A decanal solution was added to 1 ml culture for a 0.001% final concentration. Sample triplicates were contained in 350 µl polypropylene tubes and exposed to 50, 100 and 150 MPa over three 10 minutes compression and 10 minutes decompression cycles during which luciferase expression was recorded using a photomultiplier-tube directly connected to a sapphire window in the pressure vessel.

Results: Although *Escherichia coli* K12 lux grown in LB media showed slightly higher bioluminescence values than cells grown in M9 media, there was no significant effect of growth media on luciferase expression. Nevertheless, growth conditions had an effect (P < 0.05) on pressure induced bacterial inactivation, as cells grown in M9 media showed 0±0 log reductions for all pressure levels compared to 2±0 and 4±1 log reductions for 100 and 150 MPa for cells grown in LB media.

Significance: These findings suggest that prior exposure to nutrient deficient M9 growth media might have triggered a bacterial stress response that conferred cross protection against pressure. This needs to be considered for an effective and reliable use of this technology for food preservation.

P2-196 Isolation of Antibiotic-resistant Soil Bacteria from a Detroit Urban Garden

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Introduction: Environmental reservoirs, in particular soils, are widely believed to constitute an important source of antibiotic resistance. This is because many soil bacteria are capable of producing antibiotic substances and are naturally antibiotic resistant themselves. However, the nature and extent of this antibiotic resistance reservoir is yet to be determined, especially that associated with urban agriculture.

Purpose: This study was to isolate antibiotic-resistant soil bacteria from a Detroit urban garden.

Methods: A total of 21 soil samples were collected from an urban garden in Detroit in the summer of 2015. Soil bacteria were isolated using an R2A agar, supplemented with three antibiotics including ampicillin, streptomycin and tetracycline, individually, at a concentration of 15µg/ml. Bacteria were analyzed by Gram staining, 16S rRNA gene sequencing, followed by bacteria identification using 16S rRNA gene database (http://greengenes.lbl.gov). A disk diffusion test was then performed to measure the bacteria susceptibility to ampicillin, streptomycin, and tetracycline.

Results: A total of 270 soil bacteria were isolated, with the majority of isolates being Gram negative. The predominant soil bacteria identified by 16S rRNA gene sequencing were *Chryseobacterium* sp. (30.5%), *Stenotrophomonas maltophilia* (13.8%), *Sphingobacterium faecium* (8.3%), *Flavobacterium sp.*(8.3%), followed by *Xanthomonas* sp., and *Sphingobacterium kitahiroense*. Disk diffusion data showed that ampicillin resistance was common among the bacteria (90%). Streptomycin and tetracycline resistance was identified in 40% and 20% isolates, respectively.

Significance: The data will add to our knowledge of the extent of soil bacteria serving as an environmental reservoir of antibiotic resistance in the context of urban agriculture.

Poster

P2-197 Species Identification of a Gram-positive Bacterium, *Lactobacillus fermentum*, Isolated from Canned Food by Multilocus Sequence Typing

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Introduction: *Lactobacillus fermentum* is a gram-positive species of bacterium known for the production and preservation of fermented food as an acid-producing starter culture. The primary mission of FDA is to enforce the Food, Drug and Cosmetic Act and regulate food, drug and cosmetic products. FDA employs presence of pathogenic microorganisms in these products including spoilage in canned foods as one of the regulatory action criteria and to ensure that these are safe for human consumption. This surveillance study was carried out to assess the effectiveness of pathogen control in a canned food facility located in the United States.

Purpose: The major objective of this study was genetic identification of *Lactobacillus fermentum* isolated from canned food by multilocus sequence typing (MLST).

Methods: In this study, a total of nine unopened, recalled canned food jars from the same lot containing Black Bean Corn Poblano Salsa were examined initially by conventional microbiologic protocols by performing two-step enrichment followed by streaking on a selective agar. The recovered bacterial isolates were subsequently sequence characterized at *gyr*B and 16S rRNA loci at first followed by MLST using ABI 3500 XL Genetic Analyzer**.**

Results: Of the eight subsamples examined for each sample, all subsamples of one of the containers were found positive for the presence of slow growing rod-shaped, gram-positive facultative anaerobic bacteria. Species identification of these recovered bacterial isolates was done initially by our recently developed DNA sequencing protocol based on *gyr*B and 16S rRNA loci. Later, 11-loci MLST (*clp*X, *dna*A, *dna*K, *gro*EL, *mur*C, *mur*E, *pep*X, *pyr*G, *rec*A, *rpo*B, and *uvr*C) was performed. A total similarity was observed among the 8 subs at all 13 loci sequenced, and the analysis confirmed these canned food bacterial isolates to be *Lactobacillus fermentum.*

Significance: The results clearly suggested that the multilocus sequencing protocol with modified PCR conditions can provide species-identification of *L. fermentum* in the canned food monitoring program of public health importance.

P2-198 The Impact of Co-Cultivation on Growth, Expression of Virulence Genes and In Vitro Virulence Potential of *Listeria monocytogenes*

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Introduction: The interactions between *Listeria monocytogenes* and food-associated bacteria are critical for the growth of the microorganism in food environments and often related to under-detection of *L. monocytogenes* during enrichment. However limited information exists on the impact of food microorganisms on the virulence of the pathogen.

Purpose: The study investigated: i) growth, ii) expression of virulence genes and iii) in vitro virulence potential of *L. monocytogenes* in the presence of four different food-related microorganisms.

Methods: Growth of *L. monocytogenes* (ScottA) was evaluated as monoculture or in co-culture with *L. innocua, Bacillus subtilis, Lactobacillus plantarum* and *Pseudomonas aeruginosa* in Tryptic Soy Broth (10°C/10 days and 37°C/24 hours). The transcription of 9 key virulence genes (*inlA, inlB, inlC, inlJ, sigB, prfA, hly, plcA, plcB*), in addition to invasion efficiency (45 min) and intracellular growth (4 h) in Caco-2 cells, were determined for *L. monocytogenes* grown singly or in co-culture previously incubated for 3 days at 10°C or 9 hours at 37°C.

Results: Significant differences in growth between single and co-cultures of ScottA were observed when grown with *L. innocua* at 37°C or 10°C (e.g., lower final populations) and *B. subtilis* at 37°C (e.g., growth cessation after 9 h). ScottA revealed considerably increased invasion efficiency when co-cultured with *L. innocua* but attenuated, efficiency in the presence of *B. subtilis*. Intracellular growth of *L. monocytogenes* in Caco-2 cells was reduced up to 35 folds compared to monoculture, when grown in co-cultures. The key virulence genes of *L. monocytogenes* were under-expressed after co-cultivation with *B. subtilis* at both temperatures while co-cultivation with *L. innocua* at 37°C, increased the overall gene expression levels of ScottA (e.g., 7-fold increase of *prfA*).

Significance: Investigating the impact and mechanisms of microbial interactions on growth and virulence of *L. monocytogenes* expands our understanding on the survival and infection potential of the pathogen in the gastrointestinal environment.

P3-01 Relative Quantification of TAB Spoilers in AFB Ingredients

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Introduction: *Alicyclobacillus* (TAB) are acidophilic, thermophilic, gram-positive bacteria that cause spoilage of fruit juices due to endospore survival of pasteurization. Ingredient risk assessment is part of an overall strategy to prevent TAB spoilage. However, traditional culture-based methods (IFU 12) may take over a week, causing product holds or supply chain disruptions.

Purpose: In order to reduce time to result to as short as 3 h, we developed a new qPCR-based method using the GeneDisc® PCR based technology from Pall Corporation for relative quantification of TAB contamination in filterable samples.

Methods: Briefly, 105 sugar samples including superfine sugar, cane sugar and glucose syrup, were spiked with the four major TAB spoilers (*A. acidoterrestris*, *A. acidophilus*, *A. cycloheptanicus*, *A. herbarius*). The sample sizes were comprised between 10 and 200 g. Artificially contaminated samples were diluted with distilled water and filtered through polycarbonate 0.4 µm. After direct lysis on the filter, qPCR analyses were performed using the GeneDisc Plate TAB Spoilage on DNA extract which could be optionally concentrated with the Nanosep® centrifugal device 30K. This method was also tested on naturally contaminated maltodextrine samples collected before and after micro-filtration from a sugar industry.

Results: Whatever the sample size and the spiking dose, Ct values obtained with the DNA ranges and the cells ranges from pure culture and spiked sugar samples were very close, demonstrating that the method was reproducible and linear between 100 and 10,000 bacteria/sample. Applied to naturally contaminated maltodextrin samples, this method enabled to monitor the micro-filtration efficiency by showing a 4-log reduction in *Alicyclobacillus* spp. between filtrate and inlet while none of the four TAB major spoilers was detected.

Significance: With this GeneDisc method, fruit juice and ingredients producers can now increase their profitability by implementing early and rapid in-process controls and checking efficacy of their TAB contamination reduction countermeasures.

P3-02 Mapping the Changes in Sporeforming Bacteria Contamination along the Milk Production Chain from Farm to Packaged Pasteurized Milk by a Systematic Review Approach

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Introduction: Approximately one third of the produced fluid milk in the United States is lost annually. One important factor contributing to the loss is contamination with sporeforming bacteria, which can not only survive the pasteurization process, but also grow under refrigeration conditions resulting in subsequent spoilage.

Purpose: To describe the population dynamics of sporeforming bacteria and spores in milk from farm to packing plant.

Methods: A systematic review was conducted to identify and summarize primary research studies that describe the prevalence and/or concentration of spore-forming bacteria and spores at more than one production/processing point in the same study. Relevant studies are considered from countries implementing similar processing procedures as the United States without publication year restriction.

Results: Literature searches retrieved 9,778 citations, among which data were extracted from 31 relevant citations for meta-analysis. Due to variant milk sampling points recorded in citations, we standardized the sampling points by clustering similar ones as follows: on farm, during transportation, at plant before pasteurization and after pasteurization. *Bacillus cereus* was the most reported organism. Concentrations were more abundant with 582 data points for both vegetative cells and spores, compared to prevalence data with 68 points. In general, great heterogeneity was observed among studies in the contamination of milk samples. Spore concentrations remain stable until pasteurization, in a range of 0-2.5 log spores/ml. After pasteurization, spore concentrations decrease in accordance with an increase in vegetative cells.

Significance: Although considerable research has been conducted on this topic, there are limited studies to holistically describe the population dynamics of sporeforming bacteria under the current milk production system. Findings of this study can provide insights regarding steps where spore-forming bacteria could be introduced for potential effective management, as well as further research needs to increase the quality and shelf life of milk products in the United States.

P3-03 UV-C Inactivation of Bacteria and Viruses in Coconut Water

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❖ ***Developing Scientist Competitor***

Introduction: Dedicated bacterial inactivation steps are essential to minimize the risk of foodborne infections in liquid food products. UV-C irradiation is an effective means of inactivating many pathogenic organisms and is used extensively in water treatment. It acts directly on nucleic acids of the target microbe and impedes its replication. UV irradiation might be an effective method of inactivating foodborne pathogens in liquid foods.

Purpose: To assess the ability of UV irradiation to inactivate microorganisms in coconut water, a highly opaque liquid food. UV inactivation kinetics of two surrogate viruses (MS2, T1UV) and three pathogenic bacteria (*E. coli ATCC 25922*, *Salmonella* Typhimurium, *Listeria monocytogenes)* in peptone and coconut water were investigated.

Methods: Optical properties of the samples were measured using a spectrophotometer. UV-C irradiation was applied to stirred samples, using a collimated beam operating at 253.7 nm wavelength. A series of known UV doses (0 – 40 $mJ \cdot cm^{-2}$) were delivered to the samples. A balanced design with three replicates for each treatment was exposed to selected UV treatments. The samples were then double plated and cultured to determine the microbial inactivation.

Results: UV-C irradiation effectively inactivated MS2 and T1UV in the test fluids, confirming accurate dose delivery. Inactivation levels of all organisms were proportional to UV dose. At the highest dose of 40 $mJ \cdot cm^{-2}$, three pathogenic organisms were inactivated by more than 5 log ($P<0.05$). Results show that UV-C irradiation effectively inactivated viruses and pathogenic microbes in coconut water.

Significance: Fluid optics were the key controlling parameters for effective microbial inactivation. UV dose must be calculated not only from the incident UV intensity but must also consider the attenuation in the samples. This study shows that high levels of inactivation of pathogens can be achieved in coconut water, and suggests significant potential for UV treatment of other liquid foods.

P3-04 A Quantitative Microbial Risk Assessment Model for *Listeria monocytogenes* in Ice Cream

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Introduction: *Listeria monocytogenes* is a psychrotrophic pathogen and survives during freezing and for long term, posing a risk in foods such as ice creams. The potential presence of *L. monocytogenes* in ice cream concerns because this food is prescribed for patients that undertook surgeries considering ice creams are soft and "comfort" foods.

Purpose: To estimate the risk associated with consumption of ice cream contaminated with *Listeria monocytogenes* in Brazil.

Methods: The quantitative risk assessment model consisted of six modules considering the reception of raw materials, pasteurization of the milk, ice cream mixture, pasteurization of syrup, aging and the consumption scenarios. Data from literature were used to build the quantitative risk assessment model. The model was built in Microsoft Excel spreadsheet and simulated in @RISK 7 and a total of 10,000 iterations were performed using Monte Carlo method. The outputs considered the likelihood of infection per month and number of people who can be infected in a month after consuming ice cream only for elderly people and adults, due the lack of data of child and pregnant woman.

Results: The risk model predicted that the probability of infection per *L. monocytogenes* due to consumption of ice cream was 8.46 E-06, for elderly and 6.30 E-06, for adults in average. The risk of infection was 6.93 E-07 for the elderly and 5.15 E-08, for adults also in average. The model suggest that measures to ensure the microbial quality of raw materials and to avoid cross-contamination at post-pasteurization steps will result in the biggest impact on risk of infection of *L. monocytogenes.*

Significance: Data as level of contamination of raw material, the pathogen behavior during the syrup pasteurization step and refrigerated storage for all ice cream shelf life shall be generated to corroborate the assumptions used for the development of this model.

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P3-05 Fate of *Bacillus cereus* and *Geobacillus stearothermophilus* during Fermentation of Cocoa Beans as Affected by Period of Contamination

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Introduction: Spore-forming bacteria such as *Bacillus cereus* (BC) and *Geobacillus stearothermophilus* (GB) play an important role as pathogen and spoilage organisms of processed foods, respectively.

Purpose: The aim of this study was to investigate the behavior of BC and GB during fermentation of cocoa beans.

Methods: Spores suspensions of five strains (each) of BC and GB were used. The fermentation process was performed using cocoa pods (Theobroma cacao L.) in a pilot scale (2 kg beans/box) for 7 days. In a daily basis, a different fermentation box was inoculated with 3 log CFU/g of BC and GB spores. During fermentation, samples were collected for microbiological, water activity and pH determinations. BC and GB were enumerated after heat shock (70ºC/15 min and 110ºC/10 min, respectively) on Mannitol Egg Yolk Polymyxin agar (MYP) and Dextrose Tryptone agar (DTA), respectively. Yeasts (YE), lactic acid bacteria (LAB) and acetic acid bacteria (AAC) were enumerated on Potato Dextrose Agar (PDA) + 10% tartaric acid, Man-Rogosa-Sharpe (MRS) + 50mg/L natamycin and PDA + 10% tartaric acid and 50 mg/L natamycin, respectively.

Results: The counts of BC and GB remained stable during the whole period of fermentation, except in the last two days of the process, in which increase in general, approximately 2.5 log CFU/g in populations of these bacteria were observed. This might be related to the increase of pH from 4.17 to 7.42 during fermentation, while water activity remained high (0.970). The inoculation of BC and GB spores in different days did not affect the fate of BC and GB. YE, LAB and AAC presented the behavior expected during fermentation of cocoa beans.

Significance: The fermentation process does not negatively influence on the counts of BC and GB in cocoa beans and thus, contamination of cocoa beans by spore-forming bacteria should be avoided.

P3-06 The Impact of Intrinsic and Extrinsic Factors on the In Vitro Growth of *Bacillus cereus*

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Introduction: Foodborne illness resulting from the consumption of foods contaminated with pathogenic bacteria and/or their toxins has considerably heightened the public concerns about the safety of foods. Meanwhile, *Bacillus cereus* is becoming one of the most important causes of food poisoning although major foodborne pathogenic bacteria mainly include salmonellae, listeriae and certain strains of *Escherichia coli*.

Purpose: This study was performed to reduce the risk of *B. cereus* contamination by adjusting intrinsic and extrinsic factors of foods.

Methods: Three *B. cereus* strains (KCTC 3624, KCTC 1012 and KCTC 1661) of type cultures and one strain (CH3) isolated from Cheonggukjang were used in this study. The inhibitory effects of different intrinsic and extrinsic factors, such as pH (4.0-7.0), salinity (0.5-3.0 and 11.0-14.0%), temperature (26-45ºC) and additives, on the growth of *B. cereus* were examined with an automated turbidimetric system and paper disc diffusion method which measure the growth curve of *B. cereus* and the activity of antimicrobial substances, respectively.

Results: The growth of *B. cereus* was effectively inhibited at pH 4.0-4.5, and followed by pH 5.0. The growth was also significantly reduced at a higher salinity of >11.0%, but less so at a lower salinity of 0.5-3.0%. When the growth curves of *B. cereus* at different temperatures were compared, the longest lag time (LT) and lowest specific growth rate (SGR) were observed at 26ºC, which means that the strongest inhibitory effect was shown at this lowest temperature examined. Meanwhile, it was found that the growth was significantly reduced by the addition of 1% acetic acid, 1% lactic acid (among organic acids) or 10% *Schisandra chinensis* extract (among natural substance extracts).

Significance: The data showing the inhibitory effects of intrinsic and extrinsic factors on *B. cereus* could be comprehensively applied to foods for reducing the risk of *B. cereus* contamination.

P3-07 Reduction of Vegetative Cells and Spores of *Bacillus cereus* in Fermented Soybean Products by Mild Heat Treatment

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Introduction: Korean traditional fermented soybean products (Doenjang and Cheonggukjang), contain abundant nutrients but have a risk of *Bacillus cereus* contamination during a series of unit manufacturing processes, including formulation, fermentation, and packaging processes. The viable cell counts of *B. cereus* in any type of traditional fermented soybean products should not exceed the guideline level of 4 log CFU/g in Korea (Food Code, Ministry of Food and Drug Safety).

Purpose: This study was performed to establish mild heat treatment condition which could reduce the growth of vegetative cells and spores of *B. cereus* without changes of organoleptic characteristics of Doenjang and Cheonggukjang.

Methods: Three *B. cereus* strains (KCTC 3624, KCTC 1012 and KCTC 1661) of type cultures and one strain (CH3) isolated from Cheonggukjang were used in this study. The effects of different heat treatments as functions of temperature and time (40-50ºC for 10-30 min for vegetative cells; 70-80ºC for 10-30 min for spores) were examined using *B. cereus* vegetative cells and spores inoculated into phosphate buffer, Doenjang and Cheonggukjang, respectively.

Results: In phosphate buffer, Doenjang and Cheonggukjang, a heat treatment of 50ºC for 30 min showed the strongest inhibition of the viability of *B. cereus* vegetative cells, resulting in reductions of 2.78-5.98 log CFU/g (phosphate buffer), 1.29-2.00 log CFU/g (Doenjang) and 0.21-0.49 log CFU/g (Cheonggukjang), respectively, as compared to control. Meanwhile, a heat treatment of 80ºC for 30 min showed the strongest inhibition of the viability of *B. cereus* spores, resulting in reductions of 1.39-6.07 log CFU/g (phosphate buffer), 0.68-1.50 log CFU/g (Doenjang) and 0.23-0.31 log CFU/g (Cheonggukjang), respectively, as compared to control.

Significance: These data support the possibility of successful application of mild thermal treatment to fermented soybean products for reducing the risk of *B. cereus* contamination.

P3-08 Microbial Reduction in Fresh Salad Using Natural Antimicrobials Added to Active Packaging by Vapor Contact

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Introduction: Vapor contact is an alternative when essential oils (EOs) and microorganisms are placed separately in some sealed environment and therefore microbial inhibition is achieved from a distance without direct contact of the antimicrobial agent with the food. When EOs applied in vapor phase could be effective against foodborne pathogens and spoilage microorganisms at relatively lower concentrations than when applied in liquid phase, thereby causing less effect on sensory attributes.

Purpose: The aim of this work was to analyze the microbial reduction in fresh salad using natural antimicrobials added to active packaging by vapor contact throughout 15 days of storage at 8ºC.

Methods: Cinnamon and oregano EOs were incorporated to different polymer (starch or carboxymethylcellulose) film formation solutions at different concentrations (0.00, 0.25, 0.50, 0.75, 1.00, 2.00, or 4.00%), then were applied into PET packages covers and dried. A mixture of lettuce and spinach washed and rinsed were put in the boxes. Finally, complete packages were storage at 8ºC during 15 days. Mesophilic standard plate count and total coliforms were determined every three days.

Results: For both kind of films incorporated to active packaging vapor contact reaches its highest log reduction in mesophilic count (2.5 cycles) with oregano EO (2 and 4%) showed significant differences ($P<0.05$) with respect to films formulated without EO and cinnamon EO packages. For total coliforms log cycle reduction (1.5 cycles) was achieved only with oregano EO at high concentration. Salad shelf life was increased by 30%.

Significance: Starch or carboxymethylcellulose edible films incorporating Mexican oregano EO added to active packaging can reduce microbial concentration by vapor contact increasing shelf life of fresh packaged products.

P3-09 Biofilm Formation Characteristics of *Bacillus cereus* Strains Isolated from Traditional Korean Soybean Paste

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Introduction: Food spoiler, pathogenic, and spore-former *Bacillus cereus* has been well known for biofilm formation, which is closely related with phenotypes such as contamination, resistant to biocides and antibiotics, spoilage and foodborne diseases, thus, biofilm can cause a serious problem for food industry and public health.

Purpose: Taken into account, the importance of understanding biofilm formation characteristics and diversity in processed foods, the present study was conducted to investigate biofilm formation in *B. cereus* strains isolated from traditional Korean soybean paste.

Methods: Static biofilm was formed in 96-well polystyrene plate (flat bottom) in Brain Heart Infusion at 30°C and was quantified by crystal violet staining and cell enumeration of eight wild type and two reference strains.

Results: Results showed that biofilm formation quantity, spore formation, and attachment locations were highly specific to each strain. Higher number of cells was found in higher biofilm forming strains compared to weak biofilm formers. However, OD values at 595 nm for crystal violet stained biofilms were not linearly correlated with cell numbers (R^2 = 0.64, 0.35, and 0.02 for 24, 48 and 72 h, respectively), which indicates the presence of extracellular polymeric substances in the biofilms. Dense biofilm former strains also formed higher number of spores in biofilms compared to weak biofilm formers, spore formation was reached to 71% in the highest biofilm former strain even after 24 h. Interestingly, no significant differences ($P<0.05$) were in planktonic cell numbers among different strains and were 2 to 3 log CFU/well less cells compared to the number of cells in biofilms, demonstrates cell aggregation in biofilm matrix.

Significance: These results could be useful for traditional soybean paste producing industry and scientific community to ensure food safety of public health.

P3-10 Colony Morphology and Biofilm Formation by Food Spoilage Bacteria *Lactobacillus plantarum*

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❖ ***Developing Scientist Competitor***

Introduction: The lactic acid bacterium *Lactobacillus plantarum* is widely used in food manufacturing as beneficial bacterium, while it can also cause food spoilage. In its natural environment, *L. plantarum* forms biofilms, which structurally are composed of multicellular communities of bacteria, and such biofilm formation confers resistance to environmental stresses and chemical treatments. Moreover, we found two types of colony with different morphologies, wild type (WT) and mucoid variant (MV), emerge in populations of *L. plantarum* derived from environmental isolates.

Purpose: In this study, we evaluated the effects of differences in colony morphology on biofilm structure and resistance to chemical treatment in *L. plantarum*.

Methods: WT and MV cells isolated from a population of *L. plantarum* were incubated in MRS broth at 30°C for 24 h, and their biofilm structures were examined using confocal reflection microscopy and scanning electron microscopy. To evaluate the resistance to chemical treatment, the survival rates of the biofilm cells were measured by counting the numbers of colony forming units after 1 h exposure to 8% acetic acid or 15% ethanol at room temperature. The WT and MV cells were also stained with Indian ink to observe cell-associated polysaccharides (CPS).

Results: The WT formed a thin, dense biofilm, while the MV formed a thick biofilm with a lower cell density than the WT biofilm. The WT biofilm exhibited greater resistance to ethanol than the MV biofilm, whereas the MV biofilm was more resistant to acetic acid than the WT biofilm. Moreover, compared with the WT cells, the MV cells produced increased amounts of CPSs.

Significance: These results suggest that the coexistence of WT and MV cells in *L. plantarum* populations affects the biofilm structure and confers resistance to various environmental stresses and chemical treatments. These findings have important implications for the control of *L. plantarum* and food spoilage by environmental lactic acid bacteria.

P3-11 Evaluation of Temperature Management on the Microbial Quality Control of Florida Blueberries

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Introduction: Blueberries are easily damaged by rough handling and adverse temperatures. Current cooling procedures used by blueberry growers often result in delays up to 24 h, compromising quality.

Purpose: The objective of this study was to determine the best handling practices for temperature management for quality control of blueberries.

Methods: Blueberries were harvested and split into two groups. The first group was sampled from the field, pre- and post-packing lines. The second group was cooled for 24 h and then packed. Field, pre-packed and post-packed (non-cooled) samples were examined on day 0, whereas post-packed samples (both non-cooled and cooled) were examined subsequently on days 1, 7, 14 and 21. Determination of aerobic plate count (APC) on plate count agar, and yeast and molds (Y&M) on potato dextrose agar from composite samples were performed. One hundred ml of 0.1% (w/v) sterile

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peptone water was added to the sterile sample bags with ~10 g of blueberries and homogenized for 60 s. One hundred µl of each dilution was spread plated, incubated, and APC and Y&M were counted.

Results: The average initial counts of APC from field samples were 4.2 log CFU/g, 3.8 log CFU/g from the pre-packing line, 4.5 log CFU/g from the post-packing (non-cooled) line, and 4.3 log CFU/g from the post-packing (cooled) line. Those counts for the latter two lines of samples reached 4.6 log CFU/g and 4.8 log CFU/g, respectively after 21 days. The average initial counts of Y&M were 3.9 log CFU/g, 3.6 log CFU/g, 3.9 log CFU/g and 3.5 log CFU/g for field, pre-packing line, post-packing (non-cooled) line and post-packing (cooled) line, respectively. After 21 days those counts for the latter two lines of samples reached 4.1 log CFU/g and 4.2 log CFU/g, respectively.

Significance: Information obtained will be used to recommend the best temperature management to maintain postharvest quality of blueberries.

P3-12 Effect of High Pressure Processing on the Microbiological Shelf Life of Sliced Cured Turkey Breasts

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Introduction: HPP is a processing method in which high pressure is applied to packaged food products.

Purpose: In this study, ready-to-eat, vacuum packaged sliced cured turkey breasts were high pressure treated at 86,565 psi for 180 seconds at 5.5°C to evaluate the effect of high pressure processing (HPP) for shelf life extension of this product.

Methods: Two lots of the HPP treated and untreated control products were stored at 4°C for 120 days and 60 days, respectively. Over the storage period anaerobic plate counts, lactic acid bacterial counts, and yeast and mold counts were performed in triplicate on the two lots to determine microbial spoilage of the product. Additionally appearance of the product was documented over time to determine acceptability of the product over storage.

Results: Anaerobic plate counts, lactic acid bacterial counts, yeast and mold counts of the HPP treated product over 120 days were <10 CFU/g indicating the microbiological stability of the product. The color of the treated product went from a light pink tone to a whitish tone with slight purge development over the storage period. The anaerobic plate counts of the untreated product ranged from <10 CFU/ g on day 19 to >25,000,000 CFU/g on day 55 and <10 CFU/g on day 13 to 130,000,000 CFU/g on day 60 in both lots. The anaerobic plate counts of the untreated product exceeded the critical spoilage limit of 10,000,000 bacteria/g on day 45. The color of the untreated product went from a light pink tone to a lighter pink, whitish tone with slight purge development over the 60 day storage period.

Significance: The results show that HPP at 86,565 psi for 180 seconds at 5.5°C extends the microbiological shelf life of sliced cured vacuum packaged turkey breast by 75 days stored at 4°C.

P3-13 Time Temperature Indicators (TTI) Based on Chromogenic Bacterium *Janthinobacterium* sp.

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Introduction: The cold chain of perishable food products can often be disrupted by temperature abuse. Time temperature indicators (TTI) or integrators are simple and inexpensive devices that make the continuous monitoring of the time temperature history of chilled products possible throughout the cold chain. *Janthinobacterium* sp. is a chromogenic bacterium which appears purple during its growth. The time that this bacterium colony appears purple depends on the pH value of the medium where it is cultivated, temperature and atmosphere composition.

Purpose: To develop TTI for food industry based on the chromogenic ability of *Janthinobacterium* sp.

Methods: Tryptone soy agar (TSA) with pH (HCl) of: 6, 6,5, 7, 8 or 9 was surface-inoculated with 3, 4, 5 or 6 log CFU/cm^2 of *Janthinobacterium* sp. and incubated aerobically at 0°C, 5°C, 10°C and 15°C for 17, 14, 7 and 6 days respectively. Microbiological and pH measurements of TSA were performed during incubation and the growth parameters of *Janthinobacterium* sp. at different conditions, were estimated using the Baranyi model. In parallel, image analysis was employed in order to explore its potential on estimating microbiological results. The maximum growth rate (μ_{max}) was used to determine through the Arrhenius equation the activation energy (E_a) of *Janthinobacterium* sp. for different pH.

Results: The optimum growth conditions for *Janthinobacterium* sp. used in the study were 25°C and pH=7 (under aerobic conditions). Results of microbiological analysis, showed that their population reached the highest levels (9-9.5 log CFU/g) about 72-100h at 15° C and 334h at 5° C. Significant pH changes (1.5-2.5 units) were observed in TSA samples with initial pH 6 or 6.5. The E_a ranged between 27.84 Kcal/mol (pH: 9) and 21.59 Kcal/mol (pH: 7). The E_a of the microbial growth rate in food ranges from 7.8 to 28.7 Kcal/mol. The endpoint (the time at which a distinct visual color change to the final purple was observed) of the TTI at these pH (9 and 7) when they incubated at 15 °C were 118h and 92h, respectively.

Significance: Such a study could offer a new TTI in the food industry, based on the appearance of purple color of *Janthinobacterium* sp. as a signal of spoiled food product.

P3-14 Using "*Janthinobacterium* sp. Films" as a Spoilage Indicator in Food

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Introduction: *Janthinobacterium* sp. is a chromogenic violacein-producing bacterium with a distinctive purple hue. Na-alginate films, which have been studied for their properties as carriers of bioactive compounds, could be used in combination with *Janthinobacterium* sp. as spoilage indicators on food.

Purpose: To produce and apply on foods, Na-alginate films incorporated with *Janthibacterium* sp. and assess the physicochemical parameters of films that allow the appearance of purple color on the film when food spoilage occurs.

Methods: Na-alginate films without or with different concentrations of nutritional substances (yeast, meat extract, tryptophan, skimmed milk powder, cooked meet at concentrations of 0.6% and 1.2% and NaCl: 0.6% and their respective combinations) were prepared and inoculated with 2, 4 or 7 log CFU/ml *Janthinobacterium* sp. The films were applied on Tryptone Soy Agar (TSA) and on i) pork ii) minced pork iii) beef iv) minced beef, v) minced chicken, vi) ham, vii) Gouda-cheese viii) Haloumi-cheese and viii) turkey-ham. The TSA samples were stored at 5°, 10° and 25°C, whereas the food samples at 5°C. Microbiological and image analysis in addition to pH measurements took place throughout the storage.

Results: Yeast and meat extract Na-alginate films were documented as the most efficient carriers for *Janthinobacterium* sp. These films retained the viability of *Janthinobacterium* sp. in contrast to Na-alginate films with no additives where a reduction of *Janthinobacterium* sp. population (up to 2 log CFU/g) was observed during their preparation. In addition, image analysis showed that the color of yeast and meat-extract films on TSA changed into purple 2 and 4 days earlier compared to the films with no additives, at 25°C and 5°C, respectively. During storage of minced pork at 5°C, coated with yeast (0.6%) or meat-extract films (0.6%) purple color appeared in films after 6 days.

Significance: The combined use of alginate films and *Janthinobacterium* sp. could be a promising food-spoilage indicator.

P3-15 Novel Natural Phenolic Compound-based Oxygen Scavenging System for Active Food Packaging Applications

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Introduction: Oxygen is the main cause of food spoilage in terms of color change, off flavor, microbial growth, and nutrient loss. Recently use of natural based active materials are interesting area.

Purpose: An oxygen scavenging system containing a natural phenolic compound, pyrogallol with sodium carbonate, was developed and analyzed as a possible oxygen scavenger.

Methods: The effect of several parameters, including the amount of pyrogallol and sodium carbonate, relative humidity and storage temperature, on the oxygen scavenging capability were investigated

Results: The initial, glass vial headspace oxygen content (%) of 21.1% decreased to 0.26% after 8 days of storage at room temperature when the oxygen scavenging system used a 1:1 (w/w) ratio of pyrogallol (250 mg) and sodium carbonate (250 mg). Both pyrogallol and sodium carbonate were required for optimum oxygen scavenging, otherwise the oxygen scavenging ability decreased. The oxygen content (%) decreased further to 6.55% when the amount of sodium carbonate decreased from 250 to 166 mg, which yielded a 2:1 ratio. In the present study, pyrogallol (250 mg) and sodium carbonate (250 mg) had highest oxygen scavenging capacity of 51.81 ml O_2/g and an oxygen scavenging rate of 6.48 ml O_2/g*day.

Significance: The oxygen absorption kinetics rate of pyrogallol and sodium carbonate confirmed that the material has good efficiency for use as an oxygen scavenger. Results indicated that the pyrogallol based oxygen scavenging system with moisture activation can be used as an effective oxygen scavenger for low water activity food packaging applications.

P3-16 The Combination of Nisin and ε-Polylysine is Effective to Inhibit the Growth and Production of Biogenic Amines of *Enterobacter cloacae*

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Introduction: *Enterobacter cloacae* was frequently isolated from clinical samples and a wide range of foods including meat, milk, and vegetables, and they can also produce cadaverine (CAD) and putrescine (PUT) in foods that cause food spoilage.

Purpose: This study aims to evaluate the combined effects of ε-PL and nisin on the growth of *E. cloacae* in nutrient broth and chicken meat products.

Methods: The potential synergistic effects of nisin in combination with ε-PL were evaluated using the fraction inhibitory concentration index (FICI) in nutrient broth. The effect in chicken meat products was then evaluated using the following antimicrobial treatment combinations: N1 (control sample), N2 (ε-PL 0.25 g/kg), N3 (ε-PL 0.125 g/kg,), N4 (nisin 0.5 g/kg), N5 (nisin 0.25 g/kg), N6 (nisin 0.25 g/kg, ε-PL 0.125 g/kg), N7 (nisin 0.125 g/kg, ε-PL 0.125 g/kg), and N8 (nisin 0.125 g/kg, ε-PL 0.06 g/kg). The contents of biogenic amines (BAs) were measured by high-performance liquid chromatography.

Results: The combination of ε-PL and nisin generated a total synergistic effect on the *E. cloacae* strains as indicated by FICI which were ranged from 0.18 to 0.32. The final OD600 value and the putrescine (PUT) contents in the bacterial culture grown up to 48 h at 37°C treated with nisin–ε-PL, were significantly lower than those of the other groups. The bacterial counts, and the contents of TVB, putrescine (PUT), and cadaverine (CAD) of the N6 and N7 meat groups, which were treated with nisin–ε-PL, were significantly lower than those of the other meat groups.

Significance: The combination of nisin and ε-PL was more effective than application of nisin or ε-PL alone with twofold concentration to inhibit the growth of *E. cloacae* C1 and production of biogenic amines in chicken meat products.

P3-17 Thermal Inactivation Kinetics of *Sporolactobacillus nakayamae* Spores, a Spoilage Bacterium Isolated from a Model Mashed Potato-Scallion Mixture

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Introduction: *Sporolactobacillus* species have been occasionally isolated from spoiled foods and environmental sources. Thus, food processors should be aware of its potential presence and characteristics.

Purpose: The purpose of this study was to (i) compare spore formation of *Sporolactobacillus nakayamae* in four different growth media, (ii) compare first-order and Weibull models in describing the data, and (iii) calculate *z*-values for each model.

Methods: *Sporolactobacillus nakayamae* was isolated from a food model containing cooked mashed potatoes with fresh cleaned scallions added post-cooling Spores were prepared using MRS medium, reinforced clostridial medium (RCM), Glucose Yeast Peptone with 1.5 mmol/L papaverine (GYP), and All Purpose Tween medium with sucrose and bromocresol purple (APT) and diluted in PBS to 10^5-10^6 CFU/ml. Thermal inactivation was performed for different treatment times (0 -25 min) at three temperatures (70, 75, and 80°C) using 2 ml vials. Survivors were determined by plating on MRS, RCM, GYP, or APT agar. Weibull and first-order models used to describe survivor curve kinetics and thermal death times. A comparison test (ANOVA, Post Hoc test) was used to analyze the treatments on survival ratio.

Results: Thermal inactivation parameters for *S. nakayamae* spores varied widely depending upon media formulation. GYP media consistently yielded the highest D-value over the three temperatures tested. For GYP media, the *D*-values for *S. nakayamae* were in the range of 25.24±1.57 to 3.45±0.27 min for the first-order model and 24.18±0.62 to 3.50±0.24 min for Weibull model at 70 and 80°C, respectively. The *z*-values determined for *S. nakayamae* spores were 11.91±0.29°C using the Weibull model and 11.58±0.43°C using the first-order model. The Weibull model consistently produced the best fit for all the survival curves.

Significance: This study provides novel and precise information on thermal inactivation kinetics of *S. nakayamae* which will enable reliable thermal process calculations for eliminating this spoilage bacterium.

P3-18 Microbiological Evaluation and Identification of Yeast Isolated from Natural Juices and Refreshments Commercialized in the Gran Area Metropolitana of Costa Rica

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Introduction: Yeast are microorganisms strongly related to the spoilage of natural juices. Besides this spoilage capacity, the presence of *Candida* pathogenic species in natural juices has also been reported. It is important to evaluate the presence of yeast in this type of product as well as their potential resistance to fluconazole.

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Purpose: to isolate, identify and determine the susceptibility to fluconazole of yeast present in pasteurized or not fruit juices and refreshments, commercialized in the Gran Area Metropolitana of Costa Rica.

Methods: 50 samples of fruit juices and refreshments (100 non-pasteurized and 50 pasteurized) were analyzed. To each sample, a measure of pH and Brix grades was performed. Also, the Most Probable Numer (MPN) of total and thermotolerant coliforms, *Eschericihia coli* and *Staphylococcus* aureus was performed.

Yeast were quantified using oxytetracicline glucose yeasts extract agar (OGYE) incubated at 25°C for 5 days. Vitek 2 system was used for yeast identification. (BioMéreiux, France) Fluconazole resistance was determined by the broth microdilution method proposed by the European Committee of Antimicrobial Susceptibility Testing (EUCAST).

Results: pH ranged between 2.80 and 6.61 for non-pasteurized juices, and between 2.52 and 5.14 for pasteurized ones. Most non-pasteurized juices (58%) had a range between 5.1 and 10 Brix grades, meanwhile the pasteurized ones between 10.1 and 15. 15% of non-pasteurized juices presented *E. coli* and non-sample was positive for *S. aureus.* 78% of pasteurized juices presented yeast counts under 10^2 CFU/mL, contrasting with 57% of non-pasteurized juices that presented yeast count between 10^2 and 10^5 CFU/mL. *Candida* was the predominating genre isolated from pasteurized juices.

Significance: the microorganisms levels present in non-pasteurized juices are very high, partially due to a deficiency in hygienic practices. The isolation of *Candida* as the most frequent genre agrees with literature reports. Non strain was resistant to fluconazole, these bacteria come from natural environments and have not been exposed to selective pressure that might favor the development of resistance.

P3-19 DNA Barcoding Reveals Considerable Diversity of Fungi in Dairy Products

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❖ ***Developing Scientist Competitor***

Introduction: Fungi are major spoilage organisms in dairy products. However, little is known about the diversity of naturally occurring spoilage fungi in raw milk and processed dairy products.

Purpose: Using DNA barcoding data from fungi isolated from raw and finished dairy products, we demonstrate that dairy associated fungal contaminants represent a broad diversity.

Methods: Fungal organisms were isolated from raw and finished dairy products. Samples of raw milk, cheese, and yogurt were plated on Dichloran Rose Bengal Chlortetracycline agar (DRBC) for the selective isolation of fungi. 365 fungal isolates were collected for PCR amplification. Molecular typing of all isolates was performed based on the DNA sequence data of the internal transcribed spacer (ITS) region with primers ITS4 and ITS5. For species identification, ITS region sequences were blast searched against the Unite Database.

Results: Dairy associated fungal contaminants represent a broad diversity. Twenty-two genera across two phyla were isolated across raw milk, yogurt, and cheese samples. Of the fungal species isolated, 30% were *Penicillium*, 21% were *Debaryomyces*, and 11% were *Candida*. ITS sequencing is a powerful molecular tool for identification and tracking of fungal organisms, providing a far more discriminatory tool than traditional identification methods.

Significance: This study provides a baseline understanding of the types of fungi and their sources in dairy products through the use of modern molecular subtyping tools.

P3-20 Living Fungi in Sea Salts: Their Implications for Food Spoilage

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Introduction: Sea salts are produced in salterns, environments that harbor diverse fungi adapted to harsh conditions. In light of this, the prevailing assumption that sea salt is inhospitable to all microorganisms needs to be reconsidered. Sea salt may be a source of spoilage mold inoculum when used as a food ingredient.

Purpose: The purpose of this study was to quantify and identify fungi present in commercial sea salts and to assess how community composition varies among salts of different origins.

Methods: Filamentous fungi present in seven commercially available sea salts were isolated using filtration, cultured, and quantified. The isolates were identified by DNA sequencing of a barcode region. Using ordination analysis, the composition of fungal communities among salts was assessed relative to salt origins.

Results: Every sea salt contained viable fungi, ranging from 0.07 to 1.71 CFU/g salt, with significant variation among salts (P=0.021). *Aspergillus*, *Cladosporium,* and *Penicillium* were the most abundant genera isolated. Ordination analysis indicated fungal community compositions in salt were not significantly different between salts originating from the Atlantic and Pacific oceans (ANOSIM R=-0.012, P=0.46).

Significance: Many species found have been previously isolated from solar salterns, and our data suggest these fungi may survive and remain viable in salt destined for the consumer. Thus sea salt may be a previously unrecognized source of, for e.g., *Cladosporium* and *Penicillium* spoilage of cured meat products. The fungi present, including mycotoxigenic molds, are concerning and pose a spoilage risk to certain foods made with sea salt.

P3-21 Ultraviolet-C Light Effect on the Reduction of *Saccharomyces cerevisiae* on Grapefruit and Orange Juices

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Introduction: Currently, there exists a great demand of novel technologies for the pasteurization of juice in order to guarantee the quality. Ultraviolet-C (UV-C) light is a novel technology that provides a non-ionizing light with germicidal properties at 254 nm. UV-C light is capable to inactivate microorganisms due to the damage at DNA level, delaying the replication and transcription of the cell. Moreover, citric juices are products with high acidity that inhibited the microorganisms; however, *Saccharomyces cerevisiae* is capable of growth in citric products, affecting the quality of juices.

Purpose: The aim of this research was to evaluate the effect of UV-C light on *Saccharomyces cerevisiae* inactivation in grapefruit and orange juice at two different temperatures.

Methods: Commercial pasteurized grapefruit and orange juices were obtained from a local supermarket of Puebla, Mexico. Juices were inoculated with *Saccharomyces cerevisiae* (10^8 CFU/ml) and treated with UV-C light (60 min) at different temperatures (5 and 40°C). First order kinetic reduction was used to calculate the decimal reduction time. Moreover, a sensory evaluation was performed in order to know the acceptability of treated juice.

Results: Maximum microbial reduction obtained in grapefruit juice treated with UV-C light were 1.3 and 1.1 cycles for 5 and 40°C, respectively; while in orange juice were 1.2 and 1.1 cycles for the same temperatures. In grapefruit juice, temperature treatment significantly affect decimal reduction times, showing values of 45.4 ± 2.8 and 59.2 ± 3.0 min for 5 and 40°C, respectively. However, for orange juice there was not significant effect of temperature treatment, showing values of 56.2 ± 0.4 and 57.2 ± 0.9 for 5 and 40°C, respectively. Sensorial attributes indicate that juices treated at short time (10 and 20 min) were preferred by the judges.

Significance: UV-C light treatment is a suitable manner to reduce the microbial load of spoilage microorganisms; although, it is important to combine with others treatments in order to increase the microbial reduction.

P3-22 Development and Testing of a Rapid Yeast/Mold Detection Method in Yoghurt

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Introduction: Yoghurt is a popular dairy food that has nutritional benefits yet is susceptible to fungal contamination. The resulting reduced shelf life and visible colonies on the surface affect customers' perception of a safe product. Detecting yeasts and molds is complicated by the presence of lactic acid bacteria that can cause rapid technologies to incorrectly estimate the spoilage effects. This study aimed to produce a rapid method with good selectivity for yeasts and molds.

Purpose: A novel oxygen depletion technology, GreenLight, has been introduced to reduce the time to result for screening tests in dairy products. This technology uses the respiration of viable aerobic organisms to estimate microbial loads in food samples. A method was developed for introduction to dairy producers in Slovakia and the Czech Republic, with the goal of delivering good yeast/mold selectivity while minimizing sample preparation and reducing time-to-result versus the traditional microbial methods.

Methods: The study compared known fungal contaminants inoculated into fruit yoghurt, purchased at retail. Samples were tested on the rapid technology and compared to a traditional plate count method. Anti-microbials were introduced and evaluated for their improvement on selectivity of the target organisms. A customized broth was created to be used in further factory validation.

Results: Final laboratory verification results using customized broth produced 24 data pairs in triplicate for two target organisms. These indicate that the rapid system can enumerate *Candida tropicalis* at 10 CFU/ml in approximately 18 hours with correlation to plate count of 0.89 (Pearson Coefficient). This compares favorably to 5 days for the reference method. *Geotrichum candidum* was measured in approximately 22 hours (Pearson 0.96) at the same level.

Significance: The study predicts that a product release test for spoilage organisms can be designed that reduces time-to-result in packaged yoghurt products by over 100 hours, allowing early detection, recall and reduced waste.

P3-23 Rapid Detection and Characterization of Post-processing Contaminants in Conventionally Pasteurized Fluid Milk

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Introduction: Post-processing contamination (PPC) with psychrotolerant Gram-negative bacteria represents a major quality concern in conventionally pasteurized fluid milk. Reducing contamination and improving process control requires identification of the primary groups of bacterial contaminants responsible for PPC and development of methods for rapidly detecting PPC in fluid milk.

Purpose: The goal of this project was to 1) determine the primary bacterial groups responsible for PPC and fluid milk spoilage and; 2) evaluate various methods of rapidly detecting PPC in freshly pasteurized fluid milk.

Methods: Microbiological analyses were performed on 105 HTST pasteurized fluid milk samples from 20 dairy plants throughout shelf life using Coliform Petrifilm, EB Petrifilm, Crystal Violet Tetrazolium agar, and Standard Plate Count agar. Additionally, aliquots of each sample were subjected to two different testing parameters (13°C for 18 h and 21°C for 18 h) to enrich for low levels of PPC, prior to enumeration on selective media listed above.

Results: Over half (56%; n=59) of the 105 samples tested reached spoilage levels during shelf life due to PPC with gram-negative bacteria. Of these samples, non-coliform EB were responsible for under 3% (n=2) of the spoilage and coliforms for 20% of the spoilage (n=12). The remaining 76% (n=45) of the samples with PPC were shown to have spoiled due to non-EB gram-negative bacteria (i.e., *Pseudomonas*), which represented 51% of the total spoilage observed in this study. Comparison of enrichment protocols with shelf-life results indicate that 73% of the PPC could be detected within 72 h of processing.

Significance: Non-EB gram-negative bacteria represent a significant hurdle to reducing PPC in fluid milk. Rapid, accurate detection of PPC is critical in identifying contamination, minimizing food loss and producing high quality products.

P3-24 The Relationship between Socioeconomic Status and Critical Violations in Food Establishments

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Introduction: Numerous studies have investigated the impact of socioeconomic status (SES) on personal health. These studies have taken into account accessibility to different types of food markets, attitudes toward food products, and cost of food in different geographic areas. Neighborhood characteristics such as poverty and racial composition have been examined using Geographic Information Systems (GIS) to determine the correlation between these factors and obesity heart disease and diabetes. Limited research has used GIS technology to track foodborne illness rates and food safety risks.

Purpose: The purpose of this study was to use GIS technology to identify correlations between regional SES and the number of critical health code violations (CHV) found in food establishments located in the Rappahannock-Rapidan Health District of Virginia.

Methods: Data for the CHV was gathered from food establishment inspections conducted by the Virginia Department of Health during a six year period. Census tracts using natural breaks were used to divide the population within the study area into different poverty levels. Inspection reports were used from food establishments located in the areas of low and high SES. Twenty-two (22) census tracts, 187 food establishments, and 874 inspection reports were included in the final evaluation.

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Results: Analysis of Variance indicated that facilities in areas of high SES had approximately 1.7 fewer critical violations than those in areas of low SES (*P*-values <0.05). When both SES groups were evaluated together, there were 1.1 more CHV in ethnic than non-ethnic food establishments (*P*=0.03).

Significance: Socioeconomic status may impact risks for foodborne illness in retail food facilities. Targeting low SES communities for increased and customized food safety education and training through community outreach programs, schools, and social media would be a judicious use of resources.

P3-25 Food Safety Violations Observed among Mobile Food Units in Three Texas Communities

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✦ ***Undergraduate Student Award Competitor***

Introduction: Mobile food vendors are one of the fastest growing segments of the food service industry. Though typically subject to the same regulatory requirements as stationary establishments, there are limited data describing the ability of these vendors to maintain food safety during hours of operation.

Purpose: The objective of this study was to identify common food safety violations among mobile food vendors from three Texas communities.

Methods: Inspection reports of mobile food vendors from 2006 through the spring of 2015 (*n*=210) were obtained from Brazos, Harris, and Tarrant counties. Reports were coded by violation and level of compliance (1=In Compliance, 2=Not Applicable, 3=Not Observed, 4=Corrected On Site, 5=Out Of Compliance) for statistical analysis.

Results: Common violations identified included lack of soap and towels for hand washing (16%), lack of access to hand washing facilities (15%), and lack of food thermometers or the presence of thermometers that were not accurate or properly calibrated (15%). In addition, 13% of the reports identified either a lack of an approved water supply, or insufficient capacity, temperature range or pressure. More than 10% of the reports identified food contact surfaces, including equipment and utensils that were not in good repair. Cross-contamination and lack of good hygienic practices among the employees were noted in 8% and 6% of the reports, respectively.

Significance: This retrospective study of inspection reports demonstrates that mobile food vendors are not immune to the same food safety challenges that traditional food vendors face. In addition, this survey highlights unique challenges that mobile food vendors must overcome in order to reduce the risk of a foodborne illness outbreak. More research in this area needs to be conducted so food safety educators can effectively develop targeted training programs to strengthen food safety practices and mitigate consumer risk to foodborne illness.

P3-26 Will Employees Speak Out? The Impact of Training and Job Satisfaction on Approach Intention

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Introduction: Approach intention, which describes how confident employees feel about talking to others who engage in unsafe behaviors, has been identified as a successful technique to reduce levels of unsafe behaviors and could be used as an indicator of food safety culture. Hence employees with a high approach intention can be extremely helpful in reducing the risk of foodborne illness outbreaks. However, little empirical research has been conducted on investigating the factors that can improve approach intention.

Purpose: This study aimed to explore the impact of perceived training quality and job satisfaction on employees' approach intention of food safety.

Methods: A survey was designed to collect food workers input on current employment, training, job satisfaction and approach intention with regards to food safety with 1,203 responses received nationwide. A content adequacy survey was conducted to validate the survey scale. Structure equation modeling (AMOS 4.0) was used to explore the relationships among perceived training, job satisfaction, and approach intention.

Results: Adequate construct validity was proved by using confirmatory factor analysis (CFA) (χ^2= 2.67; *P*<0.01; CFI= 0.986; RMSEA= 0.041 ;). Relationships were tested by structure equation modeling (SEM). The results showed perceived training quality is positively related to job satisfaction (β = 0.545; *P*< 0.01), perceived training quality is positively related to approach intention (β = 0.122; *P*< 0.01), and job satisfaction is positively related to approach intention (β = 0.632; *P*< 0.01). The mediation effect of job satisfaction on perceived training quality and approach intention was supported by significant bootstrap test.

Significance: The results of study imply that when employees perceive high quality training and are satisfied with their job, they are more likely to make an effort to report potential food safety problems.

P3-27 Factors Associated with Employees Working while Sick

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Introduction: Transmission of pathogens from food workers to food contributes to approximately 20% of foodborne illness outbreaks. While the FDA has recommended food workers who are experiencing vomiting or diarrhea should be excluded from work, few studies have explored the factors as to why food workers still go to work while sick.

Purpose: This study identified factors (including demographic and work related factors) that contributed to food workers decisions to work despite having foodborne illness symptoms.

Methods: A national survey was designed to identify whether food workers had worked while sick, the reason why they went to work sick, perceived training quality, job satisfaction and demographic factors. Logistic regression (SPSS 22.0) was used for statistical analysis.

Results: Surprisingly, 74.7% of the respondents (N=1,203) reported they went to work when they were sick. Responses such as "I don't want to let co-workers down" (33.9%), "I can't afford to lose pay" (32.75%), and "I don't believe I would be contagious" (24.4%) were the primary reasons that food workers gave as to why they still went to work when they were sick. The logistic regression showed a good model fit (-2 log likelihood = 474.26, Cox & Snell R square = 0.427). The Baby Boomer generation (B= -0.395, *P*< 0.01), male (B= -6.98, *P*< 0.01) and lower income level (B= -1.59, *P*< 0.01) employees with lower perceived training quality (B= -0.507, *P*= 0.034) had a higher probability of going to work when they were sick compared to other identified groups.

Significance: While employees reported that they felt like they were letting their co-workers down, food safety leaders need to create a strong food safety cultures where employees realize that they truly will be letting their co-workers down by coming to work sick endangering their co-workers as well as their customers.

P3-28 Perceived Quality of Food Safety Training Based on Generation, Gender, Job Position and Education

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Introduction: Food processors, retail operations and food service companies spend thousands of dollars and man-hours training employees on proper food safety practices. Despite these efforts, foodborne illness outbreaks still occur. In order to protect consumers and businesses, it is imperative to determine what are the most effective delivery methods. Therefore, it is essential to test perceived food safety training effectiveness for food workers.

Purpose: This study examined whether demographic factors (gender, generation, job position, education level) have an effect on perceived training quality among food workers.

Methods: A national survey was designed to collect food workers' perceived training quality and demographic information with 1,203 responses. The dependent variable was perceived training quality and the independent variables were generation (Baby Boomer, Generation X, Millennials), gender, education level and job position (produce, process, and distribution), creating a 3* 2* 2* 2 study design. Statistical analysis was conducted and a multivariate analysis of variance (MONOVA) was used.

Results: For main effects, employees with a high school education perceived training quality significantly higher (4.00) than those with a college education (3.53) (F= 4.00, *P*<0.05). There was no significant difference of perceived training quality for genders, generations or job position. For interaction effects, a two-way interaction between education level and gender was significant (F-value= 3.87, *P*< 0.05). Female employees with higher educational level tended to perceive training quality lower than males. A three-way interaction among generation, gender and job position was also significant (F-value= 3.84, *P*<0.01). Generation X female employees working in the produce industry had the highest perceived training quality.

Significance: The results suggest perceived training effectiveness differed depending on the employee's education level. Therefore, food safety training materials should be task specific as well as balanced with the appropriate level of explanation of why certain food safety behaviors, tasks and procedures should be followed.

P3-29 Evaluation of Current Food Safety Practices at Various Food Establishments in Lahore, Pakistan

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❖ ***Developing Scientist Competitor***

Introduction: Food safety system in Pakistan is facing new challenges due to rapid population growth, globalization of food trade, poor sanitation, unhygienic practices and lack of consumer awareness.

Purpose: The purpose of study was to assess and evaluate the current food safety practices adopted by various food establishments and food safety knowledge and awareness among food handlers.

Methods: In present study, a cross sectional Survey of 500 food establishments of 9 towns of Lahore capital which are under jurisdiction of Punjab Food Authority was carried out in collaboration with Punjab Food Authority. Evaluations were done by structured questionnaire that was prepared as per requirements of Food Code 2013 Dubai Municipality and Food Code 2013 United States Public Health Services. The food establishments rated against criteria set by Food Standards Agency, UK. SPSS version 16 was used for all data analysis and descriptive statistics.

Results: Results of this study showed that food establishments have not properly adopted food safety practices. Thirty-six percent (36%) food premises needs immediate improvement,75% needs major improvement, only 2% food establishments have showed good sign. In personnel hygiene, 17% food establishments' needs immediate improvement and 26% require major improvement. Almost 80% food establishments require immediate improvement on account of their maintenance and infrastructure. Sixty percent (60%) food establishments require urgent improvement in response to control of operation. Conditions on fast food chains and on manufacturing industry are comparatively better.

Significance: This study is quite helpful for food establishments to improve and adopt food safety practices, to curtail the risks of food borne diseases and to minimize the health consequences associated with the consumption of unsafe food in Pakistan.

P3-30 Minimization of Cross-contamination of Gloves Used in Food-handling Applications through Surface Texturing and Functionalization

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❖ ***Developing Scientist Competitor***

Introduction: The use of superhydrophobically modified food-safe gloves in food industry is a very promising application for preventing bacterial attachment and transfer of pathogens from one surface to another surface.

Purpose: The purpose of this study was to coat the surfaces of common food-safe gloves with fluorinated silica nanoparticle (FSN) to make superhydrophobic surfaces and prevent the bacterial attachment.

Methods: Latex, nitrile, and polyethylene gloves were coated with FSN by simple one-step dip coating deposition method. The wetting characteristics of glove surfaces were determined by the measurement of static water contact angles. FSN-coated and bare gloves were dip-inoculated for 1 h and 24 h with *Salmonella* Typhimurium LT2 and *Staphylococcus aureus* at bacterial concentrations of 8.6 to 9.0 log CFU/ml. Bacterial attachment to superhydrophobically modified food-safe gloves was compared with bare gloves. Plate count method and direct counting via scanning electron microscopy (SEM) analysis were used for counting attached bacteria on glove surfaces.

Results: FSN-coated latex, nitrile, and polyethylene gloves showed superhydrophobic (Θ>160°) properties based on the static water contact angle measurements. FSN-coated latex, nitrile, and polyethylene gloves were tested against *Salmonella* Typhimurium LT2 and *Staphylococcus aureus*, and showed significantly lower (1-2 log CFU/ml) bacterial populations than bare gloves, via plate counting (*P*<0.05). SEM images demonstrated that the attachment of both bacteria to FSN-coated glove surfaces were significantly lower than bare glove surfaces (*P*<0.05).

Significance: Surface properties of food-safe gloves can be modified from hydrophilic to hydrophobic by coating with fluorinated silica nanoparticles. Coated gloves showed excellent antiadhesion properties by preventing the attachment of both *Salmonella* Typhimurium LT2 and *Staphylococcus aureus*. The coating technique used in this study can also be used in the hygienic design of food-contact surfaces.

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P3-31 Cleanliness of Environmental Surfaces in Elementary Schools as Determined by ATP Levels

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Introduction: Environmental surfaces in U.S. schools are vehicles in the transmission of microorganisms known to cause acute gastroenteritis. Students who contact contaminated surfaces then eat without washing their hands increase their risk for illness.

Purpose: Our aim was to determine the cleanliness of environmental surfaces in elementary schools as determined by ATP levels.

Methods: In Spring 2015, trained data collectors swabbed surfaces of 19 objects in cafeterias (27 observations), bathrooms (100 observations), and 3rd grade classrooms (192 observations) at five elementary schools in South Carolina for ATP revelation—expressed as relative light units (RLU)—using a close zig-zag pattern (AccuPoint Advanced ATP Hygiene Monitoring System, Neogen, Lansing, MI). In cafeterias, surfaces were swabbed before lunch; in bathrooms and classrooms, surfaces were swabbed after lunch. ATP levels on each surface were categorized as "Pass" (≤150 RLU), "Caution" (151-299 RLU), or "Fail" (≥300 RLU).

Results: The objects that failed most frequently in cafeterias were eating table tops (*n*=5; 5 failed), PIN machines (*n*=4; 4 failed), and hand sanitizer pump handles (*n*=3; 3 failed); in bathrooms were sink faucet handles (*n*=22; 3 passed, 19 failed); and in classrooms were computer keyboards (*n*=29; 2 passed, 1 caution, 26 failed). The objects that passed most frequently (or at all) in cafeterias were chair seats (*n*=5; 1 passed, 4 failed), tray rails (*n*=5; 1 passed, 4 failed), and milk refrigerator door handles (*n*=5; 1 passed, 4 failed); in bathrooms were drying devices (*n*=22; 5 passed, 2 caution, 15 failed); and in classrooms were backpack/coat storage areas (*n*=24; 11 passed, 2 caution, 11 failed).

Significance: We determined that many surfaces in our sample of five elementary schools were unclean. These findings can inform cleaning and disinfection protocols of high-risk surfaces as well as can demonstrate the importance of good hand hygiene practices among students and staff.

P3-32 Content Analysis of Vomit and Fecal Matter Clean-up Procedures to Prevent the Spread of Enteric Agents in Retail/Foodservice Operations

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Introduction: Noroviruses are the leading cause of acute gastroenteritis and foodborne disease, sickening 19 to 21 million Americans each year. A concentrated source of norovirus particles is vomit and diarrhea, illustrating the importance of proper clean up. Clean-up procedures are required in states that have adopted the 2013 Food and Drug Administration (FDA) Food Code. However, compliance could be low, particularly among the 70% of establishments independently owned and operated suggesting the need for editable model procedures.

Purpose: Our aim was to assess vomit/fecal matter clean-up procedures to determine clarity of presentation and alignment with the 2013 FDA Food Code.

Methods: In July 2015 vomit/fecal matter clean-up procedures were located by (1) asking NoroCORE (Norovirus Collaborative for Outreach, Research, and Education) stakeholders for procedures used by their constituency groups and (2) conducting a Google Advanced Search of the World Wide Web using the terms: *vomit AND fecal AND clean AND disinfect AND foodservice.* We then performed content analysis to assess clarity of presentation using the Centers for Disease Control and Prevention Clear Communication Index and to determine alignment with recommendations outlined in the 2013 FDA Food Code, Annex 3 2-501.11.

Results: A total of 40 artifacts were analyzed. The mean clarity score was 6.9±2.7 (3 to 15) of 20 points; the mean alignment score was 6.4±1.9 (2.4 to 9.9) of 11 points. Only three artifacts were classified as high clarity, high alignment. Most (24) were low clarity and high alignment.

Significance: Vomit/fecal matter clean-up procedures must align with the FDA Food Code, the best evidence base to date, and be clearly presented, yet only three artifacts met these two conditions. If we are to reduce the burden of illness attributed to noroviruses, existing clean-up procedures must be modified to improve their clarity and alignment.

P3-33 Microbial and Chemical Assessment of Campus Water Filling Stations and Water Fountains

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Introduction: Water filling stations for reusable bottles on college campuses are increasing yet have not been investigated for hygiene, microbial levels, and chemical water quality. Reusable water bottles are unique as the user dictates the cleaning regimen but can refill anywhere. Filling station conditions and reusable water bottle use may impact public health as well as food safety in foodservice due to cross-contamination.

Purpose: The purpose of this study was to enumerate microbial populations on public water fountains and filling stations to determine the influence of reusable water bottles on public water structures.

Methods: Buildings (*n*=4) with a water fountain and filling station were assessed and sampled three times over three months. Swabs were used to sample the spouts and a 10 cm^2 area on the water fountain and filling station. Water samples were taken at each location for chemical (pH, chlorine, and metals) and microbial evaluation. Dilutions were prepared using 0.1% peptone and plated to Petrifilm™ Aerobic Count Plates (APC) (48 h; 35°C) and Petrifilm™ *E. coli*/Coliform (24 h; 35°C). Two reviewers assessed the hygiene using a rubric. Student's *t*-tests were used to determine statistical differences between the fountains and filling stations (α=0.05).

Results: Filling stations had higher APC ($10.4x10^3$ CFU/cm^2) than fountains (8.8 CFU/cm^2) ($P<0.05$) in the 10 cm^2 swabbed area. Water fountain and filling station spouts and water chemistry were not different ($P>0.05$). Coliforms were present at three of four filling station sites in the 10 cm^2 swabbed area while coliforms were not found at fountain sites. Reviewers evaluated the filling stations to be less clean than water fountains ($P<0.05$).

Significance: Data suggests poor sanitation and hygiene exists at filling stations compared to fountains. The poor sanitation of filling stations and frequent reusable water bottle use may provide cross-contamination opportunities at filling stations and foodservice establishments, thus impacting public health and food safety.

P3-34 Microbial Evaluations on the Restaurant Facilities and Utilities at Hotels in Korea

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Introduction: Foodborne outbreaks occurred among guests and the employees at hotels in many countries.

Purpose: The microbial evaluations were carried out at restaurants on five first grade hotels in Busan, Korea.

Methods: Total four hundred and eight swabbed samples using sterilized sponge were collected from the surface of restaurant facilities and utensils at hotels and total, psychrotroph, and coliform counts were assessed. Fifty swabbed surface samples were also collected for the detection of presence of *Listeria* spp., *Salmonella* spp. and *E. coli* O157:H7. One hundred and sixty air samples were collected at interior spaces at four hotels for measuring counts of total, coliform, *Staphylococcus* as well as mold and yeast. All collected samples kept in an ice-packed box were moved to the laboratory and analyzed.

Results: The results gave an account that many samples were highly contaminated by general microorganisms, psychrotrophs and coliforms. The levels of contamination showed a broad distinction among sampling sites at each hotel. Total counts of samples were ranged from not detectable to 9.8×106/100 cm^2, while those of psychrotrophs and coliforms from not detectable to 7.9×106/100 cm^2 and 1.1×106/100 cm^2, respectively. *Salmonella* spp. and *E. coli* O157:H7 were detected at drains of one hotel. Microbes also detected from agar strips of air samples for total, coliform, *Staphylococcus* as well as mold and yeast counts. Heavily contaminated sites were floor, trench, rubber glove, side dish, rice cooker, dumbwaiter, cart wheel, and sink.

Significance: Therefore, these results demonstrated that those highly suspected sites should be focused and controlled according to control points of sanitation standard operating procedures.

P3-35 Yuck Factor Versus Risk Factor: What Shoppers See and Identify as Food Safety Problems at Retail

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Introduction: Food safety-related infrastructure, procedures, and practices at grocery stores play an important role in protecting public health. Retail food employees are trained to identify and minimize food safety hazards in grocery stores, but consumers often lack this training and may differ in their perceptions of food safety risk.

Purpose: Knowledge regarding consumer perceptions of food safety risk of ready-to-eat (RTE) foods in retail food establishments is lacking. The goal of this study was to understand perceptions, food safety attitudes, and self-reported behaviors related to observed food safety hazards of consumers who shop at grocery stores.

Methods: A nationally representative online survey was administered in January 2016 to 517 pre-screened participants. The survey presented questions about self-reported behavior in food safety risk situations, foodborne illness experiences, food preparation practices, and demographics. Participants were presented with 12 photos portraying i) cross-contamination, ii) temperature control, iii) hygiene, and iv) sanitation supplemented with commonly perceived food safety risks and asked to identify what they saw, whether it was safe or unsafe, and what actions they would take.

Results: Respondents identified risks factors in 6 out of 9 actual risk photos over half of the time: poor produce storage sanitation (87%, *n*=448), cross-contamination during meat slicing (85%, *n*=438), bare-hand contact of RTE food (65%, *n*=340), separation of raw and RTE food (63%, *n*=323), cross-contamination from serving utensils (60%, *n*=312), and incorrect storage temperature (51%, *n*=262). The majority (>50%) of participants also reported non-risk factor perceived risks as unsafe or very unsafe in terms of food safety.

Significance: Misperceptions of food safety risk exist among consumers. The results of this survey will be triangulated with shopper behaviors using observation methodologies and form the foundation for communication interventions for consumers and retail food safety professionals, with the goal of improved hazard and risk identification.

P3-36 Development of a Decision-making Matrix for Assessing the Shelf Stability of Cheeses

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❖ ***Developing Scientist Competitor***

Introduction: The FDA Food Code defines foods with pH <4.2 and any a_w, or a_w <0.88 and any pH as "time/temperature control for safety (TCS) foods." TCS foods require refrigeration at <5°C, or must be discarded after unrefrigerated storage for >6 h at temperature <21°C. Many cheeses are deemed TCS foods according to Food Code criteria.

Purpose: Our goal was to combine laboratory data and published research to develop a decision-making matrix that could assist industry personnel, retailers, and regulators in assessing suitability of cheeses for extended room temperature storage at retail.

Methods: One hundred two cheeses in sliced forms were challenged with single-pathogen cocktails: *Listeria monocytogenes* (10 strains), *Salmonella* spp. (6), *Escherichia coli* O157:H7 (5), and *Staphylococcus aureus* (5), at starting concentration ca. 10^4 CFU/g. Inoculated cheeses were vacuum-packaged and stored at 25°C. Samples were measured for pH and enumerated on selective agars at day 0, 3, 6, 9, 12, and 15. Un-inoculated cheeses were analyzed for % moisture, salt, and titratable acidity. Background flora of inoculated cheeses were determined on day 0, 6, and 15. Survival under temperature cycling (alternating between 4°C and 25°C at every 12 h) and aerobic storage were tested for 35 of 102 cheeses and surviving bacteria enumerated.

Results: Natural cheeses made from pasteurized cow's milk, with the exception of mold-, bacterial surface-ripened, and Swiss-style cheeses, which meet one of the requirements below, are safe for non-refrigerated storage up to 15 days, at temperatures < 25°C: i) pH ≤ 5.10, ii) pH > 5.10 and % moisture ≤ 39, iii) pH > 5.10 and % salt-in-the-moisture phase ≥ 7.20, or iv) pH > 5.10 and water activity (a_w) ≤ 0.95.

Significance: Cheese may be stored at retail for up to 15 days at up to 25°C when a letter guaranteeing cheese pH and either % moisture, % salt-in-the-moisture-phase, or a_w accompanies the cheese on shipment to retail establishments.

P3-37 Modeling the Risk of Salmonellosis Associated with Consumption of Frozen Pre-cooked Pancakes

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Introduction: Quantitative microbial risk assessment (QMRA) models are increasingly viewed as a means to help food processors make scientific decisions in support of their food safety systems.

Purpose: The purpose of this study was to assess the risk of salmonellosis associated with the consumption of minimally processed frozen pre-cooked pancakes using both laboratory data and QMRA mathematical models.

Methods: Pancake batter samples were inoculated with *Salmonella* spp. cocktails to ~10^8 CFU/g. Samples were treated at 160, 165, 170 or 175°F and from 0 to 60 seconds in a pre-heated water bath. The cooking methodology mimicked the final water activities of pancakes following cooking under commercial manufacturing conditions. The study was repeated 3 times and *Salmonella* were enumerated using scientifically valid methods. Data on pancake consumption and *Salmonella* prevalence and concentration on raw flour were based on data from published sources, internal company data or expert opinion. A QMRA model was developed using @Risk software.

Results: Cooking pancake batter to internal temperatures of 165 or 170°F reduced the average number of *Salmonella*-positive servings significantly ($P < 0.05$) compared to uncooked batter. Predicted salmonellosis cases from cooked pancakes were less than one per year when cooked to an

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internal temperature of 165°F. This number dropped by 10 fold compared to pancake batter cooked to an internal temperature of 170°F. The model estimates that a single salmonellosis case would occur about every 4.5 or 333 years when pancake batter cooked to internal temperatures of 165 or 170°F, respectively, based on a worse-case scenario (99th percentile), and the assumed parameters.

Significance: The quantitative risk analysis estimations indicate that the risk of salmonellosis from properly cooked pancake batter (where "proper" is defined as an average internal temperatures of 176°F or above, and no longer a slurry and suitable for sale) is extremely low.

P3-38 Validation of Cooking Instructions for the Reduction of *Salmonella* spp. and *Listeria monocytogenes* in Frozen French Fry Products

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Introduction: Frozen French fry products are typically considered not ready-to-eat, are sold in the foodservice markets with labelled cooking instructions, and are intended to be cooked before consumed. Currently, there is a lack of scientific literature demonstrating the lethality of labelled cooking instructions for French fry products.

Purpose: The purpose of this study was to evaluate if labelled cooking instructions for tater tots (a French fry product) are adequate to achieve an acceptable reduction in vegetative pathogens.

Methods: Frozen tater tots (not ready-to-eat) were obtained from a commercial facility. Samples were thawed, individually inoculated both on the surface and internally with multiple strains of *Salmonella* spp. and *L. monocytogenes* to achieve a target level of 10^8 CFU/g. Samples were fried directly from a frozen state in canola oil containing oil bath maintained at temperature of 350 ± 5°F (represents a worse-case scenario for cooking instructions). Frying times were between 0 and 120 seconds. Samples were analyzed for *Salmonella* spp. and *L. monocytogenes* using scientifically valid methods. The experiment was performed in triplicate. ANOVA analysis was performed to understand the inactivation kinetics of *Salmonella* spp. and *L. monocytogenes* during frying. The level of significance used was 5%.

Results: Both *Salmonella* and *L. monocytogenes* counts decreased with increase in frying time. An average of 4-log reduction (P<0.05) of *Salmonella* spp. and *L. monocytogenes* was achieved in tater tots when fried in the oil bath for 120 seconds (recommended instructions for cooking time is 120 seconds).

Significance: The results of this study demonstrate that compliance with labelled cooking instructions can result in an acceptable reduction of vegetative pathogens in French fry products. The data from this study can be used to perform scientific food safety risk assessments in the frozen French fry industry.

P3-39 Prevention of Cross-contamination during Retail Preparation of Whole and Fresh-Cut Cantaloupe

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Introduction: In a retail setting, washing of whole or fresh-cut cantaloupe with potable water or water containing sanitizer may reduce microbial load including foodborne pathogens. Cross-contamination may occur during preparation when sanitizers are not included in wash water.

Purpose: This study simulated processing in a retail setting to evaluate efficacy of water, electrolyzed water (EOW), and a commercial acid sanitizer (AS) in prevention of cross-contamination.

Methods: A whole cantaloupe was dip-inoculated with a cocktail of *Salmonella* or *L. monocytogenes* to achieve approximately 5 log CFU/cm^2. One inoculated and two non-inoculated cantaloupes were treated in 76 L of tap water, EOW (free chlorine: 50 to 60 ppm), and AS (pH≈2.8, combination of lactic acid and phosphoric acid) for 5 min. Subsequently fresh-cut cubes of flesh from inoculated and non-inoculated cantaloupe were soaked in water, EOW, or AS for 90 s. Microbiological analysis was conducted to determine possible cross-contamination during a processing.

Results: EOW treatment resulted in approximately 1.5-log reduction both in *Salmonella* and *L. monocytogenes* inoculated on the rind of whole cantaloupe, which was significantly higher than tap water treatment (P<0.05). Cross-contamination of non-inoculated cantaloupes occurred when washed with inoculated cantaloupe in tap water or AS. Cross-contamination did not occur when washing was done in EOW. During peeling and cutting, 1 to 2 log CFU/g of *Salmonella* and *L. monocytogenes* were transferred from the rind to the edible flesh of cantaloupe washed with tap water; however, cross-contamination did not occur for EOW treated cantaloupe. Subsequent treatment of mixed flesh from inoculated and non-inoculated cantaloupe demonstrated that EOW treatment reduced the likelihood of cross-contamination compared with tap water and AS. No viable *Salmonella* or *L. monocytogenes* was detected from 100 ml sample of EOW processing water.

Significance: Addition of a sanitizing agent to water used for processing of whole and fresh-cut cantaloupe in a retail setting is recommended to present cross-contamination and reduce microbial load on cantaloupe.

P3-40 The Prevalence and Characterization of *Escherichia coli* and Hygiene Indicator Bacteria Isolated from Leafy Green Produce, Beef, and Pork Obtained from Farmers' Markets in Pennsylvania

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Introduction: As farmers' markets have increased in complexity, so have the food safety risks. Microbiological assessments of farmers' market foods are needed to identify risks associated with those foods to assist educators and public health officials improve the safety of foods sold at farmers' markets.

Purpose: To assess the microbiological safety and quality of produce, beef, and pork obtained from farmers' markets in Pennsylvania through the enumeration of hygiene indicators and screening of *E. coli* isolates for select virulence genes and phylogenetic profiling.

Methods: Coliforms, fecal coliforms, and *E. coli* were enumerated from produce (n=150) and meat (n=50) obtained from farmers' markets in Pennsylvania, using the Most Probable Number method. *Listeria* spp. presence was determined using Biomerieux's Vidas system. Phylogenetic analysis of *E. coli* was performed using the Clermont method, and isolates were screened for Shiga toxin-producing (STEC)- and Extra-intestinal pathogenic *E. coli* (ExPEC)-associated genes using multiplex PCR.

Results: *E. coli* was isolated from 40% (20/50) and 18% (9/50) of beef and pork, and found in 28% (15/54), 29% (15/52), and 17% (8/46) of kale, lettuce, and spinach, respectively. *Listeria* spp. was isolated from beef (8%; 4/50), kale (2%; 1/54), lettuce (4%; 2/52), and spinach (7%; 3/46), with three isolates confirmed as *L. monocytogenes*. *E. coli* isolated from meat mainly clustered into phylogroup B1 (66%; 19/29), while produce isolates primarily clustered into phylogroups B2 (36%; 14/39) and B1 (33%; 13/39). ExPEC/STEC-associated genes present in *E. coli* (n=67) included: *fimH* (100%; 67/67) *iroN* (3%; 2/67), *hlyD* (3%; 2/67), and *eae* (1.5%; 1/67).

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Significance: The presence of *Listeria* spp. and *E. coli* on farmers' market produce and meat are strong indicators that the practices of vendors have a serious impact on the safety of the foods they sell. Farmers' market vendors may benefit from food safety training and increased public health oversight.

P3-41 Prevalence of *Salmonella* and Antibiotic-resistant *Campylobacter* in Retail Ground Beef in the United States

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Introduction: *Campylobacter* and *Salmonella* are the two most common causes of diarrheal illness in the United States. Antibiotic resistance is a public health concern and has become a focus in the food industry in the past decade.

Purpose: To determine the prevalence of *Salmonella* and antibiotic resistant *Campylobacter* in ground beef at retail across the United States.

Methods: A total of 96 ground beef samples were collected from a variety of stores across five cities in the United States during the summer months of 2015. Samples were evaluated for the presence of both *Campylobacter* and *Salmonella* after pre-enrichment using a commercial, closed-platform, real-time PCR (BAX) system. Potential positives were plated onto selective media according to organism and confirmed using latex agglutination. Positive *Campylobacter* samples were isolated and subjected to National Antimicrobial Resistance Monitoring System (NARMS) protocols to identify resistance patterns. Statistical analyses were conducted in the statistical program R to determine resistance patterns for *Campylobacter* and to compare prevalence of *Campylobacter* and *Salmonella* in ground beef.

Results: Among the 96 samples, 88.5% were positive for *Campylobacter* spp. (n=85) and 1.04% were positive for *Salmonella* (n=1). A total of 79 samples were successfully isolated, frozen and subjected to recovery methods with 64 isolates being successfully revitalized and subjected to NARMS protocols. MIC breakpoints determined resistance results as follows: 21.9% were resistant to ciprofloxacin (n=14), 39.1% were resistant to erythromycin (n=25), 51.6% were resistant to gentamicin (n=33), and 14.1% were resistant to tetracycline (n=9).

Significance: Retail is the final step before the product reaches the consumer, and it is important to consider pathogens that are present at retail. Determining a baseline for and *Salmonella* and antibiotic resistant *Campylobacter* in ground beef across the United States may contribute to future interventions and controls within the food processing chain.

P3-42 Evaluating the Impact of School Nutrition Programs Cooling Techniques on *Escherichia coli* Populations in a Commercially Prepared Chili Product

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Introduction: In preventing foodborne illness outbreaks, proper food preparation practices are especially critical in commercial settings where food products are prepared in large quantities. The third leading factor in outbreaks of school associated foodborne illness is improper or "slow" cooling. Therefore, conducting research regarding cooling methods that are both effective and feasible for preventing pathogen growth is critical to public health.

Purpose: The purpose of this study was to evaluate cooling time and combinations of cooling techniques to determine their impact on *Escherichia coli* (*E. coli*) populations in a chili product.

Methods: Chili was prepared following a recipe to meet school nutrition standards and poured into commercial serving pans to 2 and 3 inch depths, then cooled to 135-140°F before inoculation with *E. coli* (target concentration of 10^4 CFU/g). Filled pans were stored in a commercial walk-in freezer (-20°C) or placed in ice water baths in a commercial walk-in refrigerator (4°C). All pans were stored uncovered or covered, with or without an air gap and temperature of the food product was monitored every minute for 24 h. Samples were plated onto MacConkey agar at 0, 4, 8, 12, and 24 h, and incubated for 18-24 hours to enumerate *E. coli* populations.

Results: No statistically significant difference (P>0.05) in *E. coli* population was observed for time, cover, treatment (refrigerator vs. freezer) or depth. No statistically significant interactions were observed (P>0.05). These data indicate that all cooling treatments evaluated were effective at controlling *E. coli* populations in chili.

Significance: Young children are an at-risk population for severe illness and life-threatening complications from foodborne pathogens. Therefore, it is necessary to conduct research to discover and evaluate cooling methods that are effective at controlling foodborne pathogens in school lunch programs and translate these data into educational materials and trainings for food service personnel.

P3-43 Internalization of Salmonella enterica Serotype Typhimurium in Beef Products as Influenced by Vacuum Marination and Antimicrobial Interventions

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Introduction: Though successful in improving palatability, the risk of pathogen internalization propagated by vacuum-tumbled marination has been documented. As the incidence of multidrug resistance (MDR) *Salmonella enterica* serotype Typhimurium is increasing, data regarding the antimicrobial interventions and internalization of this pathogen in marinated meat products is important.

Purpose: The purpose of this study was to determine the reduction of *Salmonella* Typhimurium on beef products intended for vacuum tumbled marination using organic acid interventions. Further, this projected aimed to evaluate the internalization or translocation of *Salmonella* Typhimurium into marinated beef products following application of an organic acid.

Methods: This study evaluated the efficacy of lactic acid (4%) or buffered vinegar (2%) as surface antimicrobial interventions to reduce *Salmonella enterica* serotype Typhimurium in beef sirloin flaps (n=24) intended for vacuum tumbled marination. Likewise, the translocation of *Salmonella* Typhimurium into organic acid sprayed and marinated non-intact beef products was assessed. Beef sirloin flaps inoculated with *Salmonella* Typhimurium (10^8 log CFU/ml) were sprayed with either lactic acid or buffered vinegar prior to vacuum tumbled marination (0.35% sodium chloride and 0.45% sodium tripolyphosphate) for 30 min. *Salmonella* presence following inoculation, organic acid application, and marination (internalized *Salmonella*) were evaluated.

Results: *Salmonella* was reduced by 2.4 log CFU/cm^2 in post-inoculated surface attachment. The data indicated varied reductions and surface attachment (after vacuum tumbled marination) of *Salmonella* Typhimurium among treated beef sirloin flaps. Lactic acid (4%) spray (P < 0.0001) and buffered vinegar (2%; P < 0.0001) reduced surface populations of *Salmonella* on inoculated beef sirloin flaps prior to vacuum marination. However, reductions in surface populations were greater (~ 2 log CFU/cm^2) for lactic acid in comparison with buffered vinegar (P < 0.0001). Alternatively, the

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internalization of *Salmonella* Typhimurium following vacuum marination was not influenced (P < 0.333) by the application of a surface organic acid spray prior to marination.

Significance: These results highlight the internalization of pathogens in vacuum tumbled meat products and emphasize the importance of considering these products as non-intact. Similarly, these data confirm the efficacy and utility of interventions prior to vacuum tumbled marination yet highlight the challenge or mitigating internalization. Although surface interventions are efficacious, further research is needed to identify additional strategies to mitigate internalization of pathogens into vacuum-marinated meat products.

P3-44 Factors Affecting the Adhesion Force of Virus Determined by Atomic Force Microscopy

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Introduction: Foodborne viral diseases are strongly associated with ready-to-eat food preparation environment; however knowledge on how viruses adhere to food-contact surfaces is limited. Previously we developed a protocol for determining the virus adhesive force onto abiotic surfaces using atomic force microscopy (AFM), however the reason for a wide distribution of force measurements for different substrates was not understood.

Purpose: To identify the factors other than intrinsic properties that contribute to the strength of virus adhesion onto a substrate.

Methods: MS2 coliphage or murine norovirus was grown and purified via a multi-step foam fractionation and filtrations. AFM probes were coated with a linker and virions were conjugated to the probes. A force-distance curve from which the strength of adhesion can be directly measured was generated for each measurement. Multiple locations on glass and PVC surfaces were examined to derive adhesive forces at >100 data per sample.

Results: The adhesive force of MS2 to glass substrates showed a narrow data distribution. With the same probe and virus preparation, the force measured for PVC was consistently and statistically higher than glass (P<0.01), but had a much broader distribution. When the substrate surface was imaged, glass was found to be smooth and homogeneous, whereas numerous pores of 75-250 nm in diameter with random distribution were present on PVC surfaces. The median force measured at porous regions of PVC was 2 to 3 times higher than that at smooth areas (P<0.01). Thus, both intrinsic material properties and surface porosity/heterogeneity attributed to the variation in the virus adhesion force measurements. In addition, variation was also attributed to differences in AFM probe modification, possibly due to variation in the number of virions conjugated on the probes.

Significance: Both the material type (e.g., glass vs. PVC) and heterogeneity (porous vs. non-porous) should be considered when developing strategies for minimizing virus attachment and transmission via food-contact surfaces.

P3-45 Comparison of Different Methods of Recovering a Norovirus Surrogate from the Surface of Ready-to-Eat Foods

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Introduction: Ready-to-eat is the food category most frequently implicated in norovirus outbreaks. Since these foods are so variable in composition, there is no consensus on the best way to elute viral particles from them for analytical purposes.

Purpose: The aim of this study was to evaluate four physical methods and seven elution buffers using a norovirus surrogate on three food matrices.

Methods: The four methods consisted of different combinations of pipetting, vortex mixing, sonication and filtering the buffer through a centrifugation/filtration device (Amicon). Sliced turkey breast, strawberries and lettuce were experimentally spiked with murine norovirus. The viruses were recovered after treatment and the percentage of virus recovered from the surface was determined by real-time RT-PCR.

Results: Less than 5 % of adherent virus was recovered from strawberries regardless of method or elution buffer and the best result was obtained using an elution buffer containing beef extract. Elution buffers containing 1 mol/L NaCl were the most effective on sliced turkey breast, with the sonication + pipetting + Amicon and vortex mixing + Amicon methods providing between 20 % and 60 % recovery. The latter method combined with 1 mol/L NaCl was particularly effective on lettuce.

Significance: These results will help to guide the choice of methods and elution buffers to use for a given food matrix for the purpose of detecting norovirus during outbreaks of foodborne gastroenteritis.

P3-46 A Method for Norovirus Detection in Agricultural Water, Produce, and Hand Rinse Samples

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❖ ***Developing Scientist Competitor***

Introduction: Norovirus (NoV) was responsible for an estimated 3,500 produce-associated foodborne outbreaks between 1998 and 2008. To identify routes of NoV produce pre- and post-harvest contamination, there is a need for sensitive NoV detection methods for the agricultural environment.

Purpose: Our goal was to develop a sensitive NoV detection method for fresh produce, hands, and agricultural water that identifies sample detection inhibition.

Methods: From farms and packing facilities, we rinsed cantaloupes, jalapeños, tomatoes, and hands in a 1% peptone solution and collected agricultural water. One of each sample was spiked with NoV GI.1 or GII.2 (n=10) to optimize NoV concentration and extraction, RT-qPCR amplification, and RT-qPCR amplification inhibition analysis (using an internal amplification control [IAC]). Significant comparisons were assessed by Student's t-test.

Results: The addition of an elution step (0.15N NaCl and pH 9.0), prior to NoV concentration, improved NoV amplification - likely by promoting NoV dissociation from rinsate filth. We found no significant difference (P=0.3) in NoV yield between two polyethylene glycol (PEG) concentrations (12 and 16 g/100 ml). Though bovine serum albumin (BSA) was essential to NoV detection during PEG concentration of low turbidity samples, there was no significant difference in yield (P=0.48) between 1, 2, 3, or 4% BSA. Further, a secondary, compared to a single, PEG concentration step improved the effective volume tested by 30-50 fold. In optimizing NoV amplification, RT-qPCR primer concentrations (GI: 1200 nM forward/ 800 nM reverse; GII: 400 nM forward/ 400 nM reverse), compared to control (25 nM all primers), improved the NoV yield by 2 CTs (P=0.01). Application of an optimized IAC (GI and GII: 1 pg/µl) to 50 agricultural samples resulted in the development of an algorithm to interpret preliminary RT-qPCR results (presence/absence; confirmed/suspected).

Significance: This method can be used to identify the NoV prevalence on produce, hands and agricultural water having low virus concentrations and matrix inhibitors.

P3-47 The Influence of Four Food Matrices on Aptamer Enrichment Targeting the P-Domain of Norovirus

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Introduction: Norovirus is the leading cause of gastroenteritis and foodborne illness in the US. Low virus numbers and the inability to culture norovirus pose a challenge for norovirus detection in contaminated foods. Using aptamers as extraction tools could greatly benefit virus isolation from food, enabling the development of custom extraction procedures.

Purpose: The purpose of this study is to investigate the influence of food matrices on aptamer enrichment, to assess if food matrices present during the target binding step of SELEX will alter aptamer enrichment.

Methods: Sixteen rounds of SELEX were completed in five parallel approaches: four with food matrices (strawberries, lettuce, whole oyster, and oyster diverticulum) and one in selection buffer. After target binding of the sequence pools from the final rounds was confirmed, the sequence pools were cloned, and fifty sequences from each approach examined for similar motifs and identical sequences. Binding characteristics of the sequence pools and candidates, in the presence of each food matrix and salmon sperm DNA, were determined via filter retention assays in combination with autoradiography, using α-thrombin and TBA1 as experiment control.

Results: The sequence pools of each SELEX approach exhibited different affinities to the P-Domain, with the buffer approach exhibiting the highest and the oyster diverticula approach the lowest target binding. The 50 sequences from the final rounds of the lettuce and buffer approaches each contained 8 identical sequences, whereas the sequence pools from both oyster approaches contained 41 to 46 random sequences. The two candidates which showed specific target binding (KDs: 30 nM and 80 nM), even in presence of food matrix, were enriched in the buffer approach.

Significance: This novel investigation of the food matrix influence on aptamer enrichment and the assessment of aptamer target binding in the presence of foods helps evaluate the utility of aptamers for food analysis.

P3-48 A Predictive Growth Model of *Aeromonas hydrophila* on Chicken Breasts under Various Storage Temperatures

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Introduction: *Aeromonas hydrophila* poses a threat to poultry meat because it can grow at refrigeration temperatures. A predictive model is a useful tool to improve food safety.

Purpose: This study developed a predictive growth model of *A. hydrophila* on chicken breast as a function of storage temperature (5~40°C) using a response surface model (RSM). The model can be used for controlling *A. hydrophila* without the need for detection of the organism and may be used for controlling growth.

Methods: Culture (10^2 CFU/g) as a cocktail of A. *hydrophila* (KCTC2358, KCTC12847, and KCCM11533) was inoculated on 5-10 spots on the surface of the breast. The breasts were stored at 5, 10, 20, 30, or 40°C. The lag time and growth rate fitted to the modified Gompertz equation and the relationship of the lag time and growth rate to the growth curves was modeled using an RSM. The assessment of the RSM for the growth *A. hydrophila* was evaluated using mean square error (MSE), bias (B_f) and accuracy factors (A_f).

Results: The primary models of SGR and LT showed R^2≥0.968 using the modified Gompertz equation. The SGRs at 5, 10, 20, and 30°C were 0.195, 0.239, 0.360, and 0.500 h^{-1}, respectively. The LTs at 5, 10, 20, and 30°C were 24.04, 19.19, 1.896, and 0.748 h, respectively. Secondary models were determined by nonlinear regression: SGR = 0.15014 + 0.00769*T + 0.00013*T2, LT = 37.17741 − 2.53399*T + 0.04334*T2. The appropriateness of secondary models was validated by MSE (0.00037 for SGR, 0.00174 for LT), B*f* (1.0005 for SGR, 1.0006 for LT), A*f* (0.9995 for SGR, 0.9994 for LT), and R^2 (0.999 for SGR, 0.961 for LT). Growth data at five randomized temperatures were predicted using these models, suggesting that these models can predict the *A. hydrophila* growth on chicken breasts.

Significance: Ultimately, the models of *A. hydrophila* growth could be used for effective monitoring of *A. hydrophila*contamination on chicken breasts.

P3-49 Comparison of the Murine Norovirus-1 Inactivation in Cabbage *Kimchi* with Two Different Salinities during Storage

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Introduction: Enteric noroviruses (NoV) are occasionally detected in *Kimchi,* which is a traditional dish made of fermented vegetables.

Purpose: This study was aimed at examining the effects of two levels of salt concentrations on the survival of murine norovirus-1 (MNV-1), a human NoV surrogate, in experimentally contaminated cabbage *Kimchi* stored at 5 °C for 10 weeks.

Methods: Low (1.17 %) and normal (2.22 %) salinity cabbage *Kimchi* were stored at 5 °C for 70 days. MNV-1 (5 $\log_{10}$ plaque forming units (PFU)/mL) was experimentally inoculated in these two *Kimchi.* Ten grams (145 g of cabbage and 5 g of cabbage *Kimchi* juice) of *Kimchi* were collected from each sample at 0, 3, 5, 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70 storage days and assayed for virus titer, number of total aerobic bacteria (TAB), LAB, pH, and acidity, which affect fermentation.

Results: The titers of MNV-1 in both low (1.17 %) and normal (2.22 %) salinity cabbage *Kimchi* were significantly ($p < 0.05$) decreased with increase in storage time. The overall reduction was 1.75 logPFU/mL in normal salinity cabbage *Kimchi* and 1.24 logPFU/mL in low salinity cabbage *Kimchi.* The time required to reduce the titer by > 1 logPFU/mL in normal and low salinity cabbage *Kimchi* were 4 and 8 weeks, respectively. The pH value under both salinities significantly ($p < 0.05$) decreased until 4 weeks. The maximum acidity was 0.83 % and 0.79 % in normal and low salinity cabbage *Kimchi*, respectively, during the 10 weeks. The population of TAB and LAB reached up to 7.33 log colony-forming unit (CFU)/g as a maximum population during the storage period of 3 weeks in normal salinity cabbage *Kimchi*. However, the population of TAB and LAB in low salinity cabbage *Kimchi* reached to 6.99 and 7.04 log CFU/g at 5 and 4 weeks, respectively. Through this findings, both MNV-1 and fermentation factors such as TAB, LAB, pH, and acidity of cabbage *Kimchi* were influenced by salt concentration.

Significance: The inactivation of MNV-1 in normal salinity cabbage *Kimchi* was much faster than that in low salinity cabbage *Kimchi* because the fermentation in normal salinity cabbage *Kimchi* progressed more quickly than that in low salinity cabbage *Kimchi.*

P3-50 Bactericidal Activity of Calcium Oxide (CaO, Heated Scallop-Shell Powder) against *Listeria monocytogenes* Biofilms on Egg Shell and Stainless Steel Surfaces

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Introduction: Scallop shells are waste products of scallop harvesting in districts of Korea and Japan. When scallop shells are heated to >700°C, calcium carbonate ($CaCO_3$), the main component of scallop shells, is converted to calcium oxide (CaO), which has potent bactericidal activity. The persistence of *L. monocytogenes* biofilms on food surfaces and surfaces that contact food is the main attribute facilitating its environmental spread and subsequent contamination of ready to-eat meat products.

Purpose: This study examined the bactericidal activity of 0.05–0.50% calcium oxide (CaO, heated scallop-shell powder) against planktonic cells and biofilms of *Listeria monocytogenes* (ATCC 19113) on egg shell and stainless steel surfaces, which represent the potential major surfaces found in egg processing plants.

Methods: 0.05–0.50% CaO was used to inactivate planktonic cells and biofilms of *L. monocytogenes* ATCC 19113. Hunter colors of "L" (lightness), "a" (red/green), and "b" (yellow/blue) were used for the quality of egg. Non-linear Weibull model was fitted to determine 3-log reduction of CaO (%) for the planktonic cells and biofilms of *L. monocytogenes*on the surfaces.

Results: On both surfaces, the bactericidal activity of CaO against planktonic cells and biofilms increased over log reductions of magnitude with increasing concentrations of CaO ($p < 0.05$). On egg shell surfaces, exposure to 0.05–0.50% CaO for 1 min reduced planktonic cell numbers in cell suspensions by 0.47–3.86 log (CFU/mL) and biofilm cell numbers by 0.14–2.32 log (CFU/cm^2). On stainless steel surfaces, exposure to 0.05–0.50% CaO reduced planktonic cell numbers in cell suspensions by 0.03–4.43 log (CFU/mL) and biofilm cell numbers by 0.03–2.86 log (CFU/cm^2). The color of eggs shells, which are represented by Hunter color parameters "L" (lightness), "a" (red/green), and "b" (yellow/blue), was not changed by 0.05–0.50% CaO treatments. The concentration required to reduce bacterial cell numbers by 3 log (CR = 3) (99.9% reduction), based on fits using the non-linear Weibull model, were significantly different for planktonic cells in cell suspension (0.31% CaO), biofilms on egg shell surfaces (0.57% CaO) and biofilms on stainless steel surfaces (0.46% CaO) ($p < 0.05$). Biofilms of *L. monocytogenes* were more resistant to CaO treatment on egg shell surfaces than on stainless steel surfaces due to their surface properties.

Significance: CaO represents a promising substitute for chemical disinfectants that are currently used on eggs and in egg processing plants.

P3-51 A Custom DNA Tiling Microarray for Detection and Genotyping of Common Foodborne Viruses from Fresh Produce

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Introduction: The detection and identification of virus contaminants in food are essential for prevention and investigation of foodborne outbreaks. In the absence of an efficient cell culture system for most of the common foodborne viruses, molecular methods such as microarray analysis have been applied to detect and genotype these viruses.

Purpose: The purpose of this study was to assess the effectiveness of a custom DNA tiling microarray for detecting and identifying foodborne viruses from artificially contaminated fresh produce.

Methods: Hepatitis A virus (HAV) strain HM175/18f and norovirus (NoV) strain Minerva2006 were spiked, individually or combined, onto fresh produce (tomato or celery). Viral RNA was either co-extracted with plant RNA or extracted from eluted virus particles. The microarray, which contains overlapping short-oligonucleotide probes covering partial genomes of common foodborne viruses, was custom designed and manufactured by Affymetrix. Microarray analysis was performed following the modified Affymetrix GeneChip protocol.

Results: We employed two strategies to detect viruses from fresh produce. The first is the total RNA extraction method. Tomato slices were inoculated with HAV, and total RNA was extracted using the published protocol. Poly(A)-positive RNA, including viral RNA, was further purified with Dynabeads Oligo(dT)25 before being applied to microarray. The poly(A) purification was necessary to circumvent the interference of a large quantity of ribosomal RNAs in the total RNA preparations. The current detection limit is 1E+05 RNA copies. The second method is the elution of virus particles from celery followed by ultracentrifugation. HAV and NoV were inoculated, individually or combined, at a ratio of 1000:1. Simultaneous detection of two viruses was achievable at as low as 5E+03 copies/virus.

Significance: We demonstrate the application of a custom DNA tiling microarray for detection of common foodborne viruses from fresh produce. This method has the potential to address the increasing needs in surveillance and outbreak investigations.

P3-52 An Improved, Rapid Plate-based Assay for Estimating Human Norovirus Infectivity

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Introduction: Study of human norovirus is hindered by the lack of an in vitro cultivation method, making discrimination of virus infectivity status difficult. The ability of norovirus to bind to putative receptors (histo-blood group antigens, HBGAs) is associated with infectivity, and a receptor-binding assay can serve as a proxy for estimating that infectivity. That assay design, however, is time consuming and cumbersome.

Purpose: To develop and evaluate a rapid plate-based HBGA-binding assay for human norovirus infectivity discrimination.

Methods: A sandwich-type HBGA capture assay using neutravidin plates, biotinylated synthetic HBGAs, and antibodies was optimized for detection of GII.4 Sydney Virus-Like Particles (VLPs). It was used for infectivity discrimination of VLPs exposed to heat (60°C to 80°C for one min) or copper (0-15 min). The results were compared to a traditional HBGA binding assay that was more complex and lengthy.

Results: The improved HBGA capture assay was capable of achieving a positive/negative (VLP/No VLP) ratio of 25.3±4.9 in < 2.5 hours with a detection limit of 0.1 µg/ml VLP. The assay performed in a manner similar to that of a much longer synthetic HBGA binding assay, with binding signal nearly completely abolished after heating VLPs at 75°C for one min (94.2±3.83% signal reduction). For all treatments, the loss of signal observed for the capture assay did not significantly ($P>0.05$) differ when compared to the more established assay. The new assay did not differ significantly ($P>0.05$) to the established assay for VLPs exposed to copper surfaces, with nearly complete loss (97.5±2.8%) of binding signal after 15 min.

Significance: This new HBGA capture assay allows for evaluation of norovirus receptor binding ability in under 2.5 hours. It is significantly faster, uses less reagents than previously reported assays, and is valuable for detection of infectious norovirus remaining after application of physical or chemical control methods.

Poster

P3-53 Rapid Multiplex Detection of Norovirus in Food Samples

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Introduction: Viral foodborne disease are increasing every year. This underlines the need for simple, sensitive, fast, standardized and reliable methods in order to offer analytical solutions to food industries.

Purpose: To simplify the detection of norovirus (NoV) in food, a multiplex Q-RT-PCR was developed to identify simultaneously the 2 genogroups (I or II) of norovirus.

Methods: Primers and probes are in accordance with the specifications of the ISO/TS 15216. Optimizations have been performed to reach a sensitivity and an amplification efficiency comparable with the detection of only one norovirus genogroup per reaction. A robustness study was conducted. The detection method was validated on samples (50) previously found positive at various level of contamination for Nov GI or GII. Ready-to-use ceeramTools detection kits were produced and evaluated by 4 laboratories skilled for virus detection in food on various types of food samples.

Results: A limit of detection of 2.5 and 7 genome copies/reaction with a confidence level of 95% was reached respectively for NoV GI and GII. The robustness study demonstrated standard deviations below 0.8 for inter and intra-assays and inter manipulator variations. Using the complete workflow (sample prep, RNA extraction with NucliSens reagents, ceeramTools detection kits, GENE-UP cycler), all the previously positive food samples were found positive with the duplex detection even those with a level of contamination lower than 100 copies/25 g of food. Such results demonstrate the absence of significant difference between a simplex and a multiplex detection of NoV GI and GII.

Significance: This method allows a rapid detection of norovirus and identification of the genogroup in one reaction. The analytical costs can therefore be reduced. As more analyses can be performed for lower costs, more data one norovirus circulation and prevalence can be generated leading to a better viral food safety management. ceeramTools detection kits are also available for other targeted virus such as hepatitis A virus.

P3-54 Identification of ssDNA Aptamers with Binding Affinity to Genogroup I Human Norovirus Using a Novel Selection Process

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Introduction: Detection of human norovirus in complex sample matrices is limited by the unavailability of broadly reactive reagents. Nucleic acid aptamers have recently been used as ligands to facilitate the concentration, purification, and detection of bacteria and viruses in food.

Purpose: To select ssDNA aptamers with binding affinity for norovirus genogroup I (GI) strains using a novel Graphene Oxide (GO)-based SELEX (Systematic Evolution of Ligands by EXponential enrichment) method.

Methods: A pool of random ssDNA molecules was mixed with 100 µg GO, incubated, and the aptamer-bound GO harvested by centrifugation. The GO was then incubated with a cocktail of virus-like particles (VLPs) (representing GI.1, GI.4, GI.6, GI.7 and GI.8 strains), which acted as targets for desorption of the aptamer sequences having specific VLP binding affinity. The VLP-aptamer bound moieties, present in solution, were separated from the GO by centrifugation. The supernatant was recovered, aptamer sequences amplified by PCR, gel-purified, and the pool reprocessed for another selection round. After four rounds, enriched aptamer pools were cloned, sequenced and their secondary structure analyzed using DNA Mfold. Candidates were screened for binding affinity to 12 VLPs corresponding to GI and GII norovirus strains using an Enzyme-Linked Apatmer Sorbant Assay (ELASA).

Results: Six unique clones were obtained (AP1 through AP6) having predicted free energy (dG) (Kcal/mol) values in the range of -5.78 to -10.99. Combined sequence analysis revealed six common domains (A-F). AP4 had strong binding affinity to GI.1 and GII.2 VLPs (signal intensity ratios of 6.4±0.4 and 7.8±0.7, respectively).

Significance: GO-based SELEX is a simple platform for the isolation of aptamers without requiring target elution. To our knowledge, this is the first instance of specific GI norovirus aptamer selection using VLPs in cocktail. Future work will focus on using the aptamers in detection platforms for clinical and food/environmental sample matrices.

P3-55 FDA-*Escherichia coli* Identification (FDA-ECID) Microarray: A Pan-Genome Molecular Toolbox for Serotyping, Virulence Profiling, Molecular Epidemiology, and Phylogeny

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Introduction: Most *Escherichia coli* are non-pathogenic and some have a beneficial effect for humans. However, it is important to identify pathogenic strains for clinical diagnosis as well as food safety analysis. Current methods for the identification of pathogenic *E. coli* are either time-consuming and/or provide limited and sometimes incomplete information to make a definitive determination of the potential risk to human health.

Purpose: The purpose of this study was to describe a microarray-based assay to discriminate pathogenic and non-pathogenic *E. coli*.

Methods: A high-density custom DNA microarray was designed with informative genetic features extracted from 368 whole genome sequences (WGS) for rapid and high-throughput pathogen identification. The FDA-ECID microarray contains three sets of molecularly informative features that function together to stratify strain identification and relatedness. This includes molecular serotyping, *E. coli* pan-genome content information, and recapitulating the phylogeny of *E. coli*, the latter based on 9984 SNPs providing the most discriminatory capability. We analyzed 103 diverse *E. coli* isolates with available WGS data, including those associated with past foodborne illnesses, to determine robustness and accuracy of the array.

Results: The array was able to accurately identify the molecular O and H serotypes of all 103 isolates tested. In addition, molecular risk assessment was possible with virulence maker identifications, as exemplified with the targeted *stx* and *eae* alleles. Epidemiologically, each strain had a unique comparative genomic fingerprint that was extended to an additional 507 strains with strain-level resolution demonstrated for food and clinical examples. Finally, a 99% phylogenetic concordance was established between microarray analysis and WGS using SNP-level data for advance genome typing.

Significance: The current study confirms the FDA-ECID microarray as a powerful tool for epidemiology and molecular risk assessment with the capacity to profile the global landscape and diversity of *E. coli*.

P3-56 Next Generation Sequencing as a Novel Tool for Quality Control of Food Products: Hot dog Study

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Poster

Introduction: Product mislabeling and ingredient inaccuracies misinform consumers, pose health risks, and negatively impact the economy. DNA barcoding for species identification using Next Generation Sequencing (NGS) is a reliable tool to detect fraud, ingredient substitution, and would promote a more transparent global food supply chain.

Purpose: The objective of the study was to analyze hotdog products sold by national brands and compare their product labels and ingredient lists to the results of the analyses.

Methods: Three hundred forty-five hot dog products were purchased and were sampled for DNA extraction. Following extraction, universally accepted regions for animals, plants, and bacteria were amplified, in a 96-well format, using Polymerase Chain Reaction (PCR). PCR products were sequenced on a Next Generation Sequencing (NGS) platform to identify species in products.

Results: 14.4% of purchased products indicated ingredient substitution, unexpected ingredients, or hygienic issues. Ten percent of all vegetarian products contained meat DNA, despite no claims on the product labels. Vegetarian products also accounted for 67% of hygienic issues, such as human DNA.

Significance: The usage of NGS to identify species present in a food product can allow for a more honest food industry. Detecting ingredient and/or label accuracy will encourage transparency in the food industry and allow consumers to make informed food choices that fit a variety of lifestyles.

P3-57 Whole Genome Sequence Analysis of Staphylococcal Strains Isolated from Bakery following Food Poisoning Outbreaks

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Introduction: Staphylococcal strains (n=71) were isolated from raw ingredients, food and non-food contact surfaces following multiple investigations of a bakery linked to staphylococcal foodborne poisoning outbreaks (SFPO). Strain analysis based on antimicrobial testing, PCR, and ELISA assays revealed the presence of multi-drug resistance and enterotoxin genes. Shotgun whole genome sequencing (WGS) comparisons suggested horizontal gene transfer likely played a role in strain diversity.

Purpose: Strain evaluations were performed to determine the evolutionary relationship of strains collected during multiple SFPO.

Methods: Genome sequences were produced using the Illumina MiSeq desktop sequencer. After quality filtering, de novo assemblies were constructed for downstream analyses, which included phylogenetic estimation based on a SNP matrix and also on a concatenated alignment of core genes. Core genes and functional differences were determined based on identifying orthologous genes from PGAP annotations of the de novo assemblies.

Results: Gene concurrence was observed at 96.2%, based on WGS and PCR results. The phylogenetic relationships among the outbreak samples indicated a strong degree of population substructure where many well-supported clades were found. These clades tended to be best explained by collection date. Analyses of homologous genes revealed that the core genome was substantially smaller (n=132) than the average number of open reading frames found within each sample (n=2706). We also found that the evolutionary history of many genes was indicative of recombination/horizontal gene transfer, which provides further support for the fluidity of *S. aureus* genomes.

Significance: The recovery of enterotoxigenic multi-resistant strains and the persistence of staphylococcal strains on food industry surfaces may result in food contamination which is a serious risk for consumers. The results presented here illustrate the utility of WGS data to provide information on fine scale population genetic structure, genomic differences among phenotypically different strains, and the role that temporal dynamics has in shaping those patterns.

P3-58 Evaluation of Enriched Microflora of Raw Milk Cheese Spiked with *E. coli* O157:H7 and *E. coli* O103 Using Next-Generation Sequencing Technology

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Introduction: Between 1998 and 2014, there were 38 outbreaks in the U.S. directly linked to unpasteurized cheese resulting in 804 illnesses, 172 hospitalizations, and 3 deaths. Enrichment broths are an essential component of detection methods used to isolate bacterial pathogens from foods implicated in outbreaks. A better understanding of enrichment dynamics can inform efforts to optimize enrichment broths to increase levels of the target microbe while decreasing interfering background microflora.

Purpose: Describe microflora diversity during enrichment of raw milk cheese in order to improve detection and isolation of *Escherichia coli* from this food matrix.

Methods: Raw milk cheese was spiked with either *Escherichia coli* O157 or O103 and enriched overnight using R&F *Escherichia coli* O157:H7 Enrichment Broth or mBPWp broth. High-throughput 16S rRNA amplicon sequencing resulted in a survey of bacterial species for each treatment. Reads were preprocessed with QIIME and taxonomic assignment was performed using Resphera Insight. Reads given multiple calls (e.g., identified as *E. coli*, *S. sonnei* and *S. boydii*) were noted as "possible."

Results: All portions before enrichment were dominated by *Lactococcus lactis* at 96% and above. After enrichment, *L. lactis* and total possible *E. coli* (*E. coli* and possible *E. coli*) dominated all samples at 24 to 68% and 16 to 68%, respectively, followed by *Enterobacter* (4 to 16%) and *Shigella* (1 to 7%). Next-generation sequencing tools were able to detect and roughly measure *E. coli* introduced into cheese, with significant differences noted in *E. coli* as well as total possible *E. coli* between cheese spiked with *E. coli* O103 enriched with mBPWp and non-spiked cheese enriched with mBPWp.

Significance: This study leverages NGS technologies to describe microbial diversity of raw milk cheeses before and during enrichment. These data will be used to improve precision of broths that detect and isolate *E. coli* from this important food matrix.

P3-59 Comparative Analysis of Genomic DNA Extraction Strategies from Gouda Cheese

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Introduction: The safety and quality of cheeses can be influenced by their native microbial populations. Procedures used to determine native microbiota include PCR coupled to next-generation sequencing technology. Specific quality and quantities of microbial DNA are necessary to ensure the effectiveness of these procedures.

Purpose: The purpose of this study was to compare five DNA extraction strategies from Gouda cheese to identify a high-yield method for use in downstream procedures.

Methods: Methods for DNA extraction included (1) PowerFood® Microbial DNA Isolation Kit (MO BIO Laboratories), (2) PowerSoil® DNA Isolation Kit (MO BIO Laboratories), (3) DNeasy Blood & Tissue Kit (Qiagen), (4) DNeasy Blood & Tissue Kit with pre-processing protocol A, and (5) DNeasy Blood & Tissue Kit with pre-processing protocol B. For protocol A, cheese was homogenized with 2% sodium citrate and centrifuged. For protocol B, cheese was homogenized with a solution of 0.5% sodium chloride, 1% casitone, and 2% sodium citrate, incubated for 1 h at 37°C, homogenized, and centrifuged. DNA samples were quantitated using Qubit® technology.

Results: Method (1) produced the highest DNA yield of all five techniques at 2053±158.7 ng/g of cheese, $P<0.05$. Results of methods (2) and (3) were below the level of detection (2 ng). Methods (4) and (5) produced 618±0.8 and 186±16.4 ng/g of cheese, respectively. Therefore, pre-processing of Gouda cheese using protocol A coupled with a commercial kit produced approximately 400 ng/g of cheese more DNA than when using protocol B.

Significance: Results from this study will help to identify high-yield DNA extraction methods from Gouda cheese to be used with downstream molecular procedures.

P3-60 Genetic Identification of Botanical Species in Complex Herbal Products via High-throughput DNA Barcoding

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Introduction: Dietary supplements are facing increased scrutiny from government regulators due to systemic issues with contamination, adulteration, and outright fraud. These supplements often have dozens of ingredients making label verification difficult. Chemical profile analysis depends on the age and part of the plant being tested. Traditional Sanger sequencing can identify only one gene from one species at a time making the identification of mixed products unfeasible. These limitations can be overcome by using longer read shotgun sequencing technology to process multi-target amplicon pools.

Purpose: To develop an efficient test strategy for identifying botanical ingredients from plant species that are prevalent in commercial dietary supplements, herbal remedies, spices, and other health products.

Methods: Pharmaceutically and economically important botanical species (342) were collected and 5 barcoding genes from these plants were analyzed by high-throughput shotgun sequencing. The resulting reads were compared with public data from NCBI to determine the optimal target genes for identification. Species-specific PCR assays were developed to identify 150 plant species that are most frequently encountered in the herbal product market. Individual plant samples, mixed plant samples and commercial products were screened and the results confirmed by species-specific PCR analysis.

Results: An in-house database of ~320,000 sequences with valid species names was extracted from NCBI. All 342 botanical species sequenced were taxonomically classified using this dataset. Our analysis revealed that overall 71% of them were correctly identified to the genus or species level, and 98% were identified to the family level. Potential adulterants were detected in multi-organism and commercial samples. Species-specific PCR effectively confirmed the genetic analysis and real-time PCR provided quantitative information when needed.

Significance: It is feasible to correctly identify botanical species by sequencing pools of DNA barcodes. Limited sensitivity of identification due to inadequate coverage of species in public databases was resolved by sequencing the missing species in-house. The genetic identification of complex botanical samples will become a potent tool for verifying ingredient labels and screening for potential contaminants and adulterants in commercial products.

P3-61 GenomeTrakr Database 2015: WGS Network for Foodborne Pathogen Traceback

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Introduction: In 2012 a pilot project, called GenomeTrakr, was set up to collect whole genome sequence data (WGS) to track foodborne outbreaks. This now mature network comprises about 30 labs (public health labs and academia) that collect and publically share WGS data in real time. This high-resolution, rapidly growing database is actively being used in outbreak investigations at the state, national, and international level.

Purpose: The GenomeTrakr network demonstrates how WGS data can be used in concert with traditional epidemiology for source tracking of foodborne pathogens. Along with the paradigm shift in technology this new "open data" model allows greater transparency between public health agencies, our industry partners, academia, and international partners.

Methods: The network grew rapidly in 2015. Five new labs were added, two new surveillance efforts were added for *Escherichia coli* and *Campylobacter*, and multiple data analysis pipelines were tested. The hardware and software implemented in GenomeTrakr allowed us to compare and cluster genomes of 10s of thousands of taxa at a time. Our partner, NCBI, is currently producing daily cluster results for four pathogen surveillance efforts: *Salmonella enterica*, *Listeria monocytogenes*, *E. coli*, and *Campylobacter*, all of which are publically available.

Results: The high-resolution WGS data in concert with solid epidemiological evidence has drastically enhanced our ability to identify the food source of current outbreaks for *Listeria monocytogenes*, for which the CDC is also contributing clinical isolates in real time. Here we provide details for one of these outbreaks where WGS provided the lead in a 2015 Virginia sprout outbreak.

Significance: These results demonstrate two major contributions of GenomeTrakr: WGS as a high-resolution sub-typing tool and the global benefits of having an open data model. As the database and analysis capabilities grow GenomeTrakr will become a critical tool in helping our academic, public health and industry partners develop preventative controls to make food safer globally.

P3-62 Real-time Application of Whole Genome Sequencing of Food, Environmental and Clinical *Listeria monocytogenes* Isolates in a Virginia Investigation of Contaminated Soybean and Mung Bean Sprouts

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Introduction: High-risk foods, including sprouts, have been the focus of targeted sampling by the Virginia Department of Agriculture and Consumer Services (VDACS) Food Safety Program. In March 2015, sprouts from a Virginia processor were submitted to the Division of Consolidated Laboratory Services (DCLS) for *Listeria monocytogenes* culture.

Purpose: DCLS conducts laboratory testing of foodborne pathogens in support of local surveillance and outbreak investigations conducted by the Virginia Department of Health (VDH) and VDACS. DCLS also performs molecular subtyping by pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS) of these pathogens in support of the CDC PulseNet and FDA GenomeTrakr Networks.

Poster

Methods: Sprout samples and environmental swab composites submitted to DCLS were VIDAS LIS-screened for *Listeria* spp. Isolation of *L. monocytogenes* from screen-positive samples was performed per the FDA Bacterial Analytical Manual. *L. monocytogenes* isolates from all sources were further characterized by PFGE and WGS.

Results: *L. monocytogenes* from soybean sprouts shared the same PFGE DNA fingerprint pattern type as a listeriosis patient isolate tested by DCLS in April 2015. Both isolates were determined to be highly genetically related by WGS. Comparison of the WGS results to other clinical isolates identified two additional highly genetically related isolates from 2014. Environmental sampling at the processor identified environmental contamination with *L. monocytogenes* of the same PFGE-type, also highly genetically related by WGS, suggesting that the pathogen was a resident contaminant.

Significance: Through the VA Rapid Response Team, neighboring states receiving the product were notified and a recall of contaminated sprouts was released in May 2015. Subsequent testing of sprouts from the firm identified additional *L. monocytogenes*-contaminated product that was again highly genetically related to earlier product, environmental, and clinical isolates by WGS. Regulatory action based on these findings resulted in two additional recalls before the firm permanently closed in November 2015.

P3-63 Characterization of the Malonate Utilization Operon in *Cronobacter sakazakii* Csak O:2, Sequence Type 64 Strains Using a Custom-Designed DNA Microarray and Whole Genome Sequencing

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Introduction: Malonate utilization, is an important differential trait, recognized as being possessed by six of the seven species of the foodborne pathogen *Cronobacter* and thought to be largely absent in *Cronobacter sakazakii* (Csak).

Purpose: In this study, a diverse group of 357 *Cronobacter* strains obtained during environmental sampling of five powdered infant formula production facilities in Ireland and Germany, and 93 milk powder and cheese manufacturing facilities in the USA were analyzed. This study describes the genomic diversity and phylogenetic relationships among these strains.

Methods: Genomic DNA was isolated using a QIAcube workstation and hybridizations were performed using a previously described pan-genome DNA microarray. Whole genome sequencing (WGS) was carried out on a MiSeq platform using Nextera XT chemistry.

Results: Microarray analysis (MA) determined that 22 phylogenetically-related Csak strains possessed genes of the *C. malonaticus*-like malonate utilization operon; and all, but one strain, could utilize malonate. The strains phylogenetically grouped as a separate clade among the Csak cluster. Additionally, these strains possessed the Csak O:2 serotype and 21 of the strains were identified as sequence type (ST) ST64. The malonate-negative strain was shown by MA and WGS to lack the transcriptional regulator, *mdcR*; and its ST was ST1. WGS confirmed that the nine gene, ~7.7 kbp malonate utilization operon was located in these strains between two flanking genes, *gyrB* and *katG.*

Significance: Until this investigation, the presence of malonate-positive Csak strains that are associated with foods was underappreciated, possibly leading to misidentification when relying on phenotypic identification alone. A custom designed pan genome microarray was useful in characterizing the total genome content of these CsakO:2, ST64/ST1, malonate-positive strains; and showed for the first time that these Csak strains were highly related in total gene content, serotype and ST. This study further establishes MA as a powerful platform for genomics research of *Cronobacter*.

P3-64 Genomic Characterization of Diarrheagenic *Bacillus cereus* Isolates from Dried Foods, Dietary Supplements and Animal Feed Products Utilizing MLST Markers and Enterotoxin Genes

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Introduction: The genomically diverse *Bacillus cereus* (Bc) phylogenetic group is known to contaminate most agricultural products. Bc is responsible for causing both diarrhea and an emetic (vomiting) syndrome.

Purpose: This study describes a strategy that was developed to characterize diarrheagenic Bc isolates representing different enterotoxin and allelic profiles based on PCR, multi-loci analysis (MLA), and whole genome sequencing (WGS).

Methods: Seventy-nine Bc isolates were recovered from dried spices, infant formula, dietary supplements, and medicated animal feed. An isolate's toxin profile was determined by a multiplex end point PCR assay using primers derived from the hemolysin BL (*hbl*), nonhemolytic enterotoxin (*hhe*), cytotoxin K (*cytK*), and enterotoxin FM (*entFM*) genes. The genomic diversity of the Bc isolates was characterized using our comprehensive MLA consisting of sequences of all known MLST and enterotoxin genes. Genomes were obtained from NCBI and by using WGS on MiSeq using Nexteria XT chemistry. NCBI Blast++, MEGA6 and in-house perl scripts were applied to identify alleles of target genes.

Results: Alleles for 13 enterotoxin loci from these food and feed isolates were identified using MLA, and by PCR. Phylogenetic analysis of these sequences revealed distinct patterns of sequence polymorphisms associated with isolates of specific genome groups. Comparison of the divergence of these enterotoxin gene sequences identified certain genomic landmarks. Three recently described alleles of the *entABC* toxin were also found in these isolates.

Significance: A comprehensive strategy utilizing bioinformatics with new sequence assemblies and comparing the genome sequence analysis with endpoint PCR proved to be an effective scheme to rapidly determine genome-type and enterotoxin content of diarrheagenic isolates found in dried foods and feeds. This study also demonstrates a powerful genomic strategy for further application to the phylogenetically diverse *B. cereus* group, a prerequisite towards development of future countermeasures against this important foodborne pathogen.

P3-65 Detection of Viable *Escherichia coli* in Environmental Water Using a Combined Propidium Monoazide Staining-Real-time PCR

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❖ ***Developing Scientist Competitor***

Introduction: *Escherichia coli*, as a principal fecal indicator bacterium, is used to monitor water quality worldwide. With the detection of *E. coli* by real-time PCR (qPCR), a rapid and sensitive reflection of water quality and safety can be achieved.

Purpose: The objectives of this study were to specifically detect viable *E. coli* by targeting its *ycj*M gene in a propidium monoazide (PMA)-qPCR assay, and to investigate the specificity, efficiency, and accuracy of the assay for environmental water.

Methods: Four strains of *E. coli* isolated from animal feces, were freshly grown, combined and serially diluted for inoculating into water samples. Spiked tap water and other environmental water samples, including water from Lake of the Ozarks, Missouri River and Mississippi River, were filtered or centrifuged for cell collection. Samples were then treated with PMA, followed by DNA isolation and TaqMan® qPCR detection.

Results: For pure cultures, 5 µM PMA with a 10-min light exposure was efficient at inhibiting the amplification of DNA from 10^5 CFU /ml dead *E. coli* cells, with a detection limit of 10^2 CFU/100 ml. For tap and environmental waters collected in the winter, a higher PMA concentration of 10 µM was required and as low as 10^3 CFU/100 ml viable cells could be detected in the presence of 10^5 CFU/100 ml dead cells. For water samples collected during the summer, 10^2 CFU/10 ml viable cells could be detected in the presence of 10^4CFU/10 ml dead cells, after a 20 µM PMA treatment. Significant and strong correlations were found between the PMA-qPCR and EPA Method 1603.

Significance: With proper optimization steps to remove suspended solids in environmental water samples, the PMA-qPCR could effectively and accurately differentiate between viable and dead *E. coli* cells by suppressing the amplification of DNA from dead cells.

P3-66 AOAC *Performance Tested Method* 061503: Evaluation of the *Listeria* Environmental Detection Assay for Detecting Listeria spp. in Environmental Samples on the Atlas System

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Introduction: *Listeria* is an environmentally ubiquitous organism implicated as a major cause of human foodborne illness worldwide. *Listeria*'s long lag phase and hindered growth in the presence of competitive flora presents a challenge for rapid and reliable detection with a single 24-hour enrichment step.

Purpose: To evaluate the Listeria Environmental Detection Assay for the detection of *Listeria* spp. on stainless steel, PVC plastic and sealed concrete surfaces in an AOAC Research Institute *Performance Tested Method* study.

Methods: The method included a single 24-h enrichment at 35°C in Half Fraser broth and was compared to the USDA-FSIS MLG 8.09 culture confirmation method. Selectivity was evaluated by testing 50 target microorganisms at 10X LOD and 30 non target microorganisms at > 10e8 CFU/ml.

Results: The Listeria Environmental Detection Assay demonstrated statistical equivalence compared to the USDA-FSIS MLG 8.09 reference method for the detection of *Listeria* spp. on stainless steel, PVC plastic, and sealed concrete surfaces. The test method provided a positive result for 100% of 50 target microorganisms, and a negative result for 30 non-target microorganisms.

Significance: The Listeria Environmental Detection Assay can detect *Listeria* spp. after 24 hours of enrichment and offers a rapid, specific, and user friendly test method to monitor and limit contamination issues.

P3-67 Detection of Environmental *Listeria* spp. after 18-hour Enrichment Using Actero Listeria Enrichment Medium and the Atlas System

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Introduction: Current environmental *Listeria* detection methods prescribe a primary enrichment time ranging from 22 to 48 hours. Environmental monitoring can be particularly problematic as organisms are often in an injured state and slow to grow in primary enrichments. To address this, we comparatively evaluated enrichment times for sub-lethally injured cells using Actero Listeria Enrichment Medium (ALEM), which is designed to select for and resuscitate injured *Listeria* spp.

Purpose: To use the combination of a specialized media and the Atlas System to achieve a reduced time-to-result for detecting environmental *Listeria.*

Methods: Sponge-style environmental samples from a variety of food-processing facilities were collected and inoculated with various levels of *L. monocytogenes*. After acclimating for 24 hours at 4°C, samples were enriched with either Half-Fraser or ALEM broth. Samples were collected, in duplicate, at t = 18, 20, and 24 hours into Roka G2 Transfer Tubes via transfer of 2 ml of enrichment. Transfer tubes were then prepared for analysis by the Atlas Environmental Listeria LE Detection Assay according to the product insert on the automated Atlas instrument. Samples were analyzed via culture at the 24-hour timepoint according to USDA MLG 8.09.

Results: Across spike levels, Atlas detection of *Listeria* after 18 and 20 hours of enrichment was 98 and 100% concordant, respectively, with detection via cultural methods at 24 hours in samples enriched with ALEM, Half-Fraser enrichment under the same conditions was 98% concordant at 20 and 24 hours.

Significance: Most assays for detecting *Listeria* spp. in environmental samples, including the Atlas Listeria Environmental Detection Assay, are designed to do so using non-proprietary media with a 24 to 48 hour enrichment time. For laboratories seeking more flexibility in their workflows, or simply a shorter time-to-result, the combination of ALEM and the Atlas system provides a viable alternative.

P3-68 An Independent Evaluation of a Real-time PCR Assay Including a Free DNA Removal Step for the Detection of *Listeria* Species in Select Food and Environmental Surfaces

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Introduction: *Listeria's* ability to survive in extreme 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. While less frequent than other food borne pathogens, outbreaks from *Listeria monocytogenes* have been linked to a variety of food types, such as raw milk cheeses, pasteurized dairy products, smoked seafood, deli meats, hot dogs, and cantaloupe. The presence of other *Listeria* species, such as *L. innocua, L. welshimeri or L. ivanovii* is often used as an indicator for the possible contamination of *L. monocytogenes.* The Bio-Rad iQ-Check® Listeria spp. Kit is based on gene amplification and detection by real-time PCR. Ready-to-use PCR reagents contain oligonucleotides (primers and probes) specific for *Listeria* species, as well as DNA polymerase and nucleotides. The Free DNA Removal Kit inhibits the amplification of target DNA from non-viable cells.

Purpose: The purpose of this independent evaluation was to compare the new method, including the Free DNA removal step, to the USDA-FSIS 8.09 method for deli ham (25g), stainless steel (1 x 1 swabs) and sealed concrete (4" x 4" sponges) environmental surfaces and the AOAC993.12 method for cheddar cheese (125 g)as part of the AOAC RI™ PTM validation process.

Poster

Poster

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 Bio-Rad Listeria Special Broth, test portions were evaluated by both the new and reference methods. Samples were confirmed following procedures outlined in the USDA/FSIS-MLG8.09 or AOAC993.12.

Results: Results for the assay were compared to the MLG and AOAC reference methods by POD analysis. No statistically significant differences were observed between the new method and the reference methods in the 2 foods and 2 environmental surfaces.

Significance: The data from the study, within the statistical uncertainty, support the product claims of the iQ-Check® *Listeria* spp. Kit and enhanced sensitivity in detection of *Listeria* species using the Free DNA removal protocol in the select food matrices and environmental surfaces analyzed.

P3-69 Evaluation of a Simplified Yeast and Mold Method for a Variety of Foods and Environmental Sponge Samples

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Introduction: Foods and food production surfaces are evaluated for yeast and mold contamination to assess both sanitation and product shelf life. Peel Plate YM (Yeast and Mold, PP-YM) is a self-wicking pre-dispensed 47mm diameter plating method that utilizes a chromogenic substrate for phosphatase detection in a potato dextrose formulation to detect fungal contaminants as blue/green colonies in 3 to 5 days.

Purpose: Independently evaluate PP-YM in comparison to the FDA BAM Chapter 18 method using, dichloran rose bengal chloramphenicol (DRBC) agar, in milk, mixed fruit and environmental sponge samples.

Methods: Milk (2% fat), mixed fruit (100% fruit juice) and stainless steel surfaces were artificially contaminated with fungal strains in both the manufacturer and independent laboratory, Q laboratories Inc. Target contamination levels for spiked samples at low, medium and high levels were split into *n*=5 replicates. All samples were decimally diluted and tested in duplicate. Foods were homogenized (25 g/225 ml) in 0.1% peptone water (PW), and neutralizing broth sponge-surface-swabs were homogenized with PW. The candidate method tested 1 ml of sample dilution and the standard method tested 0.1 ml from dilutions. PP-YM plates were evaluated at 72 h and 120 h incubation at 25°C, and DRBC plates after 120 h at 25°C.

Results: Colony counts (CFU/ml or g) were evaluated for repeatability (S_r) and by paired-*t*-test for statistical difference, defined as 95% confidences outside 0.5 log the reference method. The PP-YM after 5 days incubation was not-significantly different at the 95% level from the FDA-BAM at most levels of the fruit, surface evaluations and milk. Milk at some of the spike levels was an exception in the manufacturer's laboratory.

Significance: PP-YM detected fungal contamination similarly to the reference method (25°C for 3-5 days) in food and surfaces tested. PP-YM offers a new testing alternative for fungi detection in foods and food production surfaces.

P3-70 Performances Assessment of the TEMPO Technology According to the ISO 16140-2 Standard for *Bacillus cereus* Enumeration in a Broad Range of Foods and Environmental Samples

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Introduction: TEMPO is an automated technology combining TEMPO cards and selective media to ensure rapid enumeration of quality indicators. Within the TEMPO panel of methods, TEMPO BC is dedicated to *B. cereus* group enumeration in food and environmental samples.

Purpose: An independent study was conducted at Adria, to validate this method in comparison to the ISO 7932 standard, as part of the MicroVal approval process and according to the ISO 16140-2 standard.

Methods: The relative trueness study, the accuracy profiles and the limits of quantification were determined for 5 food categories and environmental samples. The inclusivity and exclusivity testing was run with 50 target strains from the *B. cereu*s group and 33 non target strains. The inter-laboratory study was run with 17 different laboratories.

Results: One hundred sixty-four samples were analyzed in the relative trueness part, 101 provided interpretable results. The data of the alternative and the reference methods were analyzed with the Scatter and Bland-Altman plots, and only few differences higher than 0.5 log CFU/g were observed leading to 3 positive and 8 negative deviations. The 6 accuracy profiles are within the acceptability limits defined in the ISO 16140-2 standard, i.e., 0.5 log CFU/g. Whatever the tested matrix, the quantification limit is 0. All the tested *B. cereus* group strains were correctly enumerated. Within the non-target strains, only one *Lysinibacillus fusiformis* strain on the three tested ones was detected by the alternative method giving a very low result, i.e., 2 log less than the control medium. The statistical calculations done with the ring trial data clearly showed that the tolerance interval limits of the alternative method are within the acceptable limits of 0.5 log.

Significance: The performance criteria are fulfilled for all the ISO 16140-2 study parts, demonstrating that the TEMPO® BC method is equivalent to the ISO 7932 standard for *B. cereus* enumeration in a broad range of foods and environmental samples. Moreover, TEMPO® BC offers important economic savings by reducing time to result and handling time.

P3-71 Performance Assessment of the VITEK MS to Confirm Characteristic Colonies after Screening for *Cronobacter* spp. Detection with ESIA One Day

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Introduction: VITEK® MS is an automated microbial identification system that uses MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-of-Flight) technology. In few minutes, this mass spectrometry technology can provide clear confirmation of characteristic colonies after a first screening for *Cronobacter* spp detection with ESIA One Day.

Purpose: An independent study was conducted at Adria to validate VITEK MS as a confirmation method according to the NF Validation technical rules and approval process.

Methods: One hundred and fifty two *Cronobacter* spp strains and 100 gram negative non-target strains, mainly from the *Enterobacteriaceae* family, were tested. The *Cronobacter* spp. strains were enriched according to the ESIA One Day protocol. The non-target strains were cultured in a non-selective broth, i.e., BHI. All the strains were streaked on ESIA selective agar, as well as non-selective TSA plates. Isolated colonies were tested for confirmation with the VITEK MS.

Results: One hundred *Cronobacter* strains were correctly confirmed as *Cronobacter* spp., "no result" outputs were obtained with the VITEK MS for 4 of the target strains. None of the non-target strains were confirmed as *Cronobacter* spp., 63 of them were not able to recover on ESIA.

Significance: Due to its robust database, VITEK MS is an accurate and rapid technology to confirm ESIA One Day positive presumptive results.

P3-72 Comparison of Manual Assurance GDS and Assurance GDS PickPen PIPETMAX Procedures for Preparation of Food and Environmental Samples

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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 method has been customized to perform an automated alternative to prepare samples for analysis.

Purpose: To compare the equivalence of sample preparation for 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 foods by multiple of methods.

Methods: A total of 556 samples of various matrices were analyzed by multiple 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 556 samples were tested. Four hundred and thirty-one (431) samples were confirmed positive with both the manually processed and those prepared using the PPMX automated system. One hundred twenty-five (125) samples were negative by both methods. There were no discrepant results. The mean Ct values across all the positive curves were within 0.2 of each other. Seven different detection assays were evaluated. In total, 22 different foods and two surfaces were tested.

Significance: This validation study demonstrates the equivalence of the evaluated sample preparation method comparing manual processing with the new automated system, the Assurance GDS PickPen PIPETMAX.

P3-73 Evaluation of Performance and Workflow Efficiency of MilliporeSigma Readybag Buffered Peptone Water Acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP for *Salmonella* Detection in Food

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Introduction: Nutrient rich Buffered Peptone Water (BPW) for *Salmonella*detection according to ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP supports high resuscitation rates and intensive growth. A phosphate buffer system prevents bacterial damage from media pH changes. This composition has been incorporated into a pouch system of pre-weighed, gamma-irradiated dehydrated media for adding directly to weighed food samples and hydrating with sterile water, reducing sample preparation time by up to 50%.

Purpose: To evaluate performance and workflow efficiency of the Buffered Peptone Water ready-to-use pouch system.

Methods: Four foods were inoculated with 4 *Salmonella* strains at ~1.0 CFU/ml, 10 replicates and enriched according to ISO 6579. Competitor comparison studies evaluated growth rate and buffering capacity by inoculating 4 *Salmonella*strains into the pouch system and 4 competitor BPW. Bioscreen C equipment measured growth rate by automating turbidity measurements at 36.5°C over 18 h. Buffering capacity was measured by recording pH of BPW media from t=0 and after addition of 200 µl 1N HCL until acidity target (pH 2.0 – 3.0) was reached at 18.0 ml. Workflow efficiency was evaluated in an independent study comparing the pouch system to high efficiency and traditional sterilizing methods for 375 g food samples.

Results: The pouch system growth rate was stronger than all 4 competitor BPW by 30% - 633%, pH decreased at the slowest rate from pH 7.0 to pH 4.0 whereas all 4 competitors decreased further to pH 2.0 – 3.0, food testing results were equivalent to ISO 6579 reference and workflow total costs were up to $210.45 cheaper and up to 3.2 h faster.

Significance: Readybag Buffered Peptone Water acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP demonstrates a stronger growth rate and superior buffering capacity compared to all 4 competitors, is equivalent to ISO 6579 reference method and improves lab efficiency by reducing labor time and cost.

P3-74 Validation of MilliporeSigma MAS-100® VF Active Air Sampler to Support Preparation of an Environmental Monitoring Program for FSMA Compliance

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Introduction: The Food Safety Modernization Act (FSMA) requires an Environmental Monitoring Program to be incorporated into the Food Safety Plan (FSP) if environmental air comes into contact with food during processing. The testing method must produce repeatable and reproducible results. Active air samplers can meet this criteria by producing good biological and physical efficiencies. Product testing according to ISO 14698-1:2003 Annex B guideline also ensures reliable fulfillment of this requirement.

Purpose: To evaluate the active air sampler performance according to ISO 14698-1:2003 Annex B guideline.

Methods: Study was conducted by an independent expert public laboratory, using Trypticase Soy agar ICR+ media plates on tested and reference systems. Physical efficiency: *Bacillus atrophaeus* spores in Potassium Iodide (KI) solutions were aerosolized and sampled. KI concentration (0 to 7% (w/v)) determined average particles size in a range of 1 µm to 6 µm mass mean diameter. Result expressed as recovery percentage of colonies counted on tested system versus reference membrane impaction air sampler. Biological efficiency: mixed suspensions of *B. atrophaeus* (BA) spores and *Staphylococcus epidermidis* (SE) cells were aerosolized and sampled. Result expressed as recovery percentage of counted colonies ratio SE/BA sampled by the active air sampler versus the ratio obtained on the reference Casella slit sampler.

Results: Physical efficiency (ten repeats per size): 84.2±14.8% at 1 µm mass mean diameter, 85.8±11.2% at 1.3 µm, 94.2±10.9% at 2.2 µm and 99.7±9.7% at 5.4 µm. Biological efficiency (twenty repeats): 76.48±26.42%.

Significance: Physical efficiency indicates high recoveries at all sizes. Biological efficiency indicates effectiveness at sampling cells without significant loss of viability. Results demonstrate that the MAS-100 VF is an efficient solution for capturing bacteria-laden air particles repeatably and reproducibly, and could be incorporated into the Environmental Monitoring Program of the FSMA Food Safety Plan for reliable environmental air sampling.

P3-75 Evaluation of Romer Labs' AgraStrip Tree Nut Assays and a Multi-tree Nut Strip for Environmental Surface Testing

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Introduction: Nut allergy affects roughly 1% of the U.S. population, with tree nuts and peanuts causing a higher percentage of life-threatening allergic reactions than other food allergens. Because trace amounts of nuts still provoke allergic reactions, highly sensitive tree nut detection methods are a critical component of food allergen risk management.

Purpose: This study was undertaken to compare detection of tree nut residues on environmental surfaces, swabbed and tested by AgraStrip (AS) tree nut assays and a multi-tree nut strip.

Methods: Tris-buffered saline was used to make protein extracts of defatted and ground almond, cashew, pistachio, walnut, hazelnut and pecan, which were then analyzed by BCA assay for protein content. Protein concentration was converted to whole allergen and used to calculate spikes at and near the stated limits of detection (LOD). Tree nut extracts were spiked onto stainless steel coupons, dried, and tested according to the instructions in each kit.

Results: The multi-tree nut assay stated a LOD of 5-20 µg for all six tree nuts based on validation of tree nut extracts spiked onto a Teflon surface and swabbed. This study showed that for stainless steel the multi-tree nut assay LOD's were 15 µg for hazelnut, 20 µg for cashew and almond, 25 µg for pistachio and walnut, and 30 µg for pecan. AS Cashew/Pistachio showed LOD of 1 µg for cashew and 2 µg for pistachio. AS Walnut (cross-reacts with pecan) had a LOD of 5 µg for walnut and 200 µg for pecan. AS Hazelnut had a LOD of 2 µg, while AS Almond had a LOD of 1 µg.

Significance: Single and dual tree nut strips demonstrated lower LOD's than the multi-tree nut strip for 5 out of 6 tree nuts, showing them to be the more sensitive method for rapid detection of trace levels of tree nut residue.

P3-76 Production and Characterization of Monoclonal Antibodies to Pork Fat Protein

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Introduction: Meat and food products adulterated with pork fat are a concern for Islamic consumers whose dietary laws prohibit consumption of pork meat. In addition, undeclared pork adulterated in meat products causes unexpected problems such as allergy and contamination of pathogenic bacteria and parasites. However no immunoassay for the rapid and simple detection of pork fat adulterated in meat and food products has been reported.

Purpose: The objectives of this study are to develop and characterize monoclonal antibodies (MAbs) specific to pork fat using thermal-stable soluble proteins as an immunogen and to detect pork fat by indirect ELISA.

Methods: Thermal-stable soluble protein extracted from pork fat was used as an immunogen. The mice showing high titer were used for cell fusion and cloning. The characterization of MAbs produced from hybridoma cells obtained were confirmed by indirect ELISA and Western blot. Laboratory adulterated pork fat in other animal species (beef, chicken, duck, goat, turkey) were prepared and analyzed by indirect ELISA.

Results: Seven MAbs (2B8-3, 2B8-9, 2B8-20, 2B8-28, 2B8-31, 2B8-32 and 2B8-33) were developed. All MAbs were specific to pork fat without cross-reaction to pork meat and other animal species in the indirect ELISA and Western blot analyses. The ELISA assay can sensitively detect 1% pork fat protein in other animal species.

Significance: These results support that the application of ELISA could be used as rapid means to detect low levels of pork fat in meat and food products.

P3-77 Reliability of Selective Media Used to Isolate and Identify *Vibrio vulnificus* and *Vibrio parahaemolyticus* from Food and Environmental Samples

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Introduction: Selective media is often used to isolate pathogens from food and environmental samples. *Vibrio vulnificus* (Vv) and *Vibrio parahaemolyticus* (Vp), the leading bacterial causes of mortality and morbidity, respectively, in United States shellfish consumers are commonly isolated using TCBS, CPC+, VVA and/or CHROMagar.

Purpose: Selective media is formulated to promote growth of desired bacteria while inhibiting others. However, how effective is the media that is relied upon for isolation and identification of Vv and Vp? This study addresses the reliability of four common media used for isolation and identification of Vv and Vp.

Methods: Suspect Vv and Vp isolates from direct plating of oyster, water, and sediment were replica plated onto T1N3, TCBS, CPC+, VVA, and CHROMagar to determine colony morphology. Colonies from T1N3 plates were inoculated into APW in 96-deep well plates and identified using multiplex PCR.

Results: Of the 2,318 isolates tested, 1,069 were confirmed via PCR as Vv and 43 were confirmed as Vp. TCBS was used to isolate and identify possible Vv or Vp. Use of this media led to correct identification of 85.80% of isolates, with a false negative rate of 24.58%. CPC+ and VVA were used to isolate and identify possible Vv. Correct identification occurred using these media with 83.14% and 70.84% of isolates, with false negative rates of 17.33% and 18.51%, respectively. CHROMagar was used to isolate and identify Vv and Vp. Isolates were correctly identified with this media 81.38% and 7.58% of the time, with false negative rates of 15.68% and 0.90%, respectively.

Significance: The use of reliable media that produces fewer false negatives supports a more accurate risk estimate. Thus, correctly identifying pathogens from food and environmental samples is important for public safety.

P3-78 Preparation and Application of Diethylstilbestrol-imprinted Magnetic Molecularly Polymers Based on the Sol-Gel Method

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Introduction: Diethylstilbestrol (DES) residue in food is in trace levels, the purification and enrichment of it from food sample is time-consuming. As a fresh process of solid-phase extraction (SPE), magnetic matrix dispersive solid-phase extraction has been taken as a powerful method to separate and enrich target components from food matrix, which overcomes demerits of SPE, and enhances the efficiency of diffusion and mass transfer highly.

Purpose: In this study, a new magnetic molecularly imprinted polymer was prepared to be used as matrix-dispersive solid phase material to extract a target in food.

Methods: The magnetic molecularly imprinted polymers (MMIPs) were prepared by the sol-gel surface molecularly imprinted technique. Preparing conditions were optimized and the prepared materials were characterized by Scanning Electron Microscope, Fourier Transform Infrared Spectroscopy, and the adsorption kinetics experiment, equilibrium binding experiment and competitive adsorption experiment had been done. The prepared

materials was used as the magnetic matrix-dispersive solid phase extraction adsorbent and coupled with HPLC for determination of diethylstilbestrol residues in milk.

Results: The test results indicated that the saturated adsorption amount of MMIPs for diethylstilbestrol was 522.50 µg g^{-1} and relative selectivity coefficient of MMIP were 3.35 of hexestrol and 2.98 of dienestrol. The optimized extraction conditions of MMIP were as following: the pH of the extraction solvent was 6.0, eluent was 3 ml methanol and acetic acid (8:2, v/v) and elution time was 3 min. The detection limit (S/N=3) of the developed analytical method could reach 2.0 µg L^{-1}, under the optimum condition, the recoveries of DES at three spiked levels ranged from 90.5% to 103.5% with Relative Standard Deviation in the range of 4.7% to 6.9%.

Significance: The established method promised potential of practical application for selective and sensitive determination of diethylstilbestrol residue in foodstuff samples, which is useful for regulators concerned about foodstuffs.

P3-79 Practical Improvement in the Detection and Enumeration of Microbial Colonies on Membrane Filters by Using a Fully Automated Microbial Detection System Based on Time-lapse Shadow Image Analysis

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Introduction: Microbial colonies on/in agar plates can be detected at their initial stage and counted precisely with MicroBio µ3D™ AutoScanner (Microbio Corporation), a fully automated microbial detection system based on time-lapse shadow image analysis, which identifies only growing spots with three-dimensionally convex shadow gradient as microbial colonies. Although membrane filter (MF) method is preferably used in beverage industry to inspect a trace of contaminant in a large volume of liquid sample, the interference of light penetration by MF often causes critical disturbance.

Purpose: The main purpose of this study is to reduce the time for detecting slow-growing bacteria with the system by using a semi-transparent MF.

Methods: Cell suspension of a wild strain of *Mycobacterium* sp. was prepared with PBS and approximately 100 cells were filtrated with either a white MF made of cellulose-mixed ester (ADVANTEC, pore size 0.45µm) or a semi-transparent MF made of polycarbonates (Millipore, pore size 0.4 µm). These MFs were placed on 1/10 Trypticase Soy Agar (TSA) plates and incubated at 28°C. Microbial colonies on the MFs were counted by the system every 30 minutes or operator's daily observation and we compared the time for detecting 90% of the final counts observed at 120 h.

Results: When a white MF was used, it took more than 96 h in operator's observation and 108 h with the system, while we succeeded in reducing the time to 70 h by replacing it with a semi-transparent MF which became almost transparent when it got wet. It should also be emphasized that manual counts often involved misjudgment of foreign particles as small colonies especially at the early time of incubation, while no miscounting has occurred with the system.

Significance: We expect that non-human operations of microbiological testing with the system will achieve both rapid results and relief of operator's tension.

P3-80 Matrix Interactions on the Detection of Milk and Peanut Residues Using ELISA

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Introduction: Reduced recoveries of allergens have been determined in processed glutinous food matrices when analyzed by enzyme-linked immunosorbent assays (ELISA). Interactions occurring in the food matrix due to thermal and non-thermal processing may be inhibiting detection of allergens by ELISA. However, it is unknown if reduced detection is resultant of processing effects or matrix interactions occurring prior to processing.

Purpose: The purpose of this study was to examine interactions occurring between unprocessed food matrices and allergenic residues, milk and peanut, using commercial ELISAs and describe the type(s) of interaction causing reduced detection.

Methods: Model food matrices containing non-fat dry milk (NFDM), wheat flour, wheat starch, salt, shortening, and water were prepared with varying levels of flour (100, 80, 60, 40, 20, 0% flour), substituting flour with starch when necessary. Analogous non-glutinous matrices incurred with NFDM were prepared without shortening and water (flour, starch, salt). Secondly, samples of a known allergen (NFDM or peanut) concentration and increasing concentrations of flour (0, 25, 250, 2500, 25000, 250000, 300000, 350000, 400000, 450000, 500000, and 550000 ppm) were prepared. Neogen Veratox Total Milk or Neogen Veratox Peanut ELISA kits were used for analysis.

Results: Statistically significant differences ($P<0.05$) were found between the recovery of milk from glutinous and non-glutinous matrices; recovery was lower in glutinous matrices (77 ± 19%) compared to non-glutinous matrices (117 ± 19%). In the concentration analyses, recovery of milk was lowest (66 ± 15%) at 550,000 ppm flour whereas recovery of peanut was adequate (98 ± 11%).

Significance: Reduced recoveries of allergenic residues by ELISA, particularly milk, are observed in glutinous food matrices and in high flour concentrations. Inadequate detection of residues does not imply reduced allergenicity therefore further understanding of the interaction between allergenic proteins and glutinous food matrices is needed to assure the safety of allergic consumers.

P3-81 Comparison of Surface Sampling Methods for Detecting Some Pathogens on Food Contact Surfaces

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Introduction: Contamination of foodborne pathogens is of major concern in the food industry. There is a strong link between the production environment and finished product quality. The most common technique used for environmental monitoring in a processing line is swabbing.

Purpose: To measure the impact of swab-type and surface on swabbing effectiveness.

Methods: Four types of swab ([1] cotton; [2] polyesterurethane foam (PU foam); [3] sponge; and [4] gauze swab) were used to recover pathogens (*Salmonella* Typhimurium, *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*) from stainless steel and polyesterurethane (wet and dry) coupons. Additionally, the efficiency of bacterial release from the different swab types was evaluated by inoculating 5 log CFU/ml of a bacterial suspension directly onto each swab type and measuring the amount of bacteria released.

Results: Sponge, PU foam and gauze swab showed higher release efficiency (average range of 4 pathogens: 94.09-99.34%) compared to cotton swab (80.76-87.17%). Swabbing on a wet surface using sponge (80.12-98.19%), PU foam (87.30-98.54%) and gauze (77.93-100.78%) yielded no significant difference of recovery efficiency, but the cotton swab was significantly lower (79.01-92.49%). For swabbing on dry surfaces, the sponge showed the highest recovery efficiency (52.31-78.40%), while cotton swab exhibited the lowest recovery (33.85-63.31%). Swabbing on dry surfaces decreased bacterial recovery efficiency of all swab types to 33.85-78.40%. The efficiency of each swab to recover bacterial biofilm from surfaces was also determined. Sponge (47.68-54.97%) and PU foam (48.29-55.16%) showed higher percentage recovery of bacterial biofilm than cotton (45.10-50.09%) and gauze swab (48.29-55.16%).

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Significance: The results of this study clearly show that swab and surface types and conditions can affect bacterial recovery efficiency. Therefore, choosing appropriate swab-types can increase bacterial recovery efficiency from surfaces and achieve more accurate estimations of the actual contamination.

P3-82 Carbohydrate Ligands as Antibody-mimics for the Expedient Extraction of *Salmonella* Enteritidis, *E. coli* O157:H7 and *Bacillus cereus* in Fresh Milk

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❖ ***Developing Scientist Competitor***

Introduction: Rapid detection of bacterial contamination in the food supply chain is critically important for food safety monitoring. A reliable capture of the bacteria from complex matrices is required to achieve high detection sensitivity especially for low contamination or for bacteria with low infective dose. Carbohydrate ligands that attach to specific cell-surface molecules of pathogens are promising substitutes of antibodies for cell targeting.

Purpose: The objective of this study was to identify carbohydrate ligands that could be used for efficient cell capture.

Methods: Two carbohydrate ligands, F#1 and F#2, were immobilized individually onto magnetic nanoparticles (MNP) which allowed easy suspension in the food matrix and expedient extraction after microbial capture. Following FDA's Bacteriological Analytical Methods, 25 ml of milk samples initially added with 5 mg MNP were inoculated with varying dilutions of bacterial stock culture. MNP and bacteria were allowed to interact briefly to form MNP-cell complexes and then separated from the milk matrix by applying a magnet to the side of the milk bottle. After supernatant removal, the complexes were re-suspended in 1 ml of fresh milk and plated per BAM procedures. Capture was carried out in vitamin D, 2% and fat free milk for the two different functional ligands and for three bacteria, *Salmonella* Enteritidis, *E. coli* O157:H7, and *Bacillus cereus*, with at least three replicates for each sample and control. All experiments were repeated over a period of several weeks to account for environmental variations. Total number of experiments (n) included in the analysis is greater than 20.

Results: Capture efficiency (CE) for all combinations of milk, functional ligands and bacteria ranged from 73% to 89%. Long-term exposure of the MNP in milk did not markedly influence CE.

Significance: Results show that F#1 and F#2 have the potential as antibody substitutes for bacterial extraction prior to detection.

P3-83 Performance of Three Methods for the Recovery of Yeast and Molds on a Variety of Products

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Introduction: Yeasts and Molds are important quality indicators in the food and beverage industry, impacting product spoilage and shelf life. The Traditional method requires five days for time to results and is difficult to interpret. 3M™ Petrifilm™ Rapid Yeast and Molds (RYM) provides results in less than 60 hours and may help in faster product releases.

Purpose: Performance of three methods for the recovery of Yeast and Molds on a variety of products

Methods: Four groups were evaluated: processed meat, dairy products, processed food and environmental samples. Thirty samples per matrix were analyzed. Samples were artificially contaminated with a mix of *Aspergillus niger* (50-100 CFU/sample) and *Saccharomyces cerevisiae* (50-100 CFU/sample). Samples were diluted and plated on 3M RYM and Mold, 3M™ Petrifilm™ Yeast and Mold (YM) and Potato Dextrose Agar. Samples were incubated for 48 hours for RYM and 120 hours for YM and PDA. **Results** were analyzed using a paired *t*-test at 95% of confidence.

Results: A total of 1,080 samples were analyzed. Recovery of molds was as follows: 2.30 logs CFU for 3M Petrifilm RYM, 2.32 log CFU for 3M Petrifilm YM and 2.17 log CFU for Potato Dextrose Agar. Media recovery of yeast was 3.02 log CFU for 3M Petrifilm RYM, 2.97 logs CFU for 3M Petrifilm YM and 2.52 log CFU for the traditional method. Statistical differences were not found between the three methods ($P>0.05$).

Significance: 3M Petrifilm Rapid Yeast and Mold and 3M Petrifilm Yeast and Mold Petrifilm Plates provide reliable and accurate results and can be used for Yeast and Mold enumeration in finished products such as dairy, processed meats, processed foods as well as environmental sampling.

P3-84 Use of 3M Molecular Detection Assay for the Detection of *Salmonella* spp. from Dehydrated Products

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Introduction: *Salmonella* is widely recognized as a foodborne pathogen. *Salmonella* has been linked to products with low water activity. Traditional methods used for the detection of *Salmonella* are labor intensive with typical time to result of 4-5 days. Fast and accurate pathogen detection in low water activity products is critical for effective food safety management.

Purpose: Use of 3M Molecular Detection Assay for the detection of *Salmonella* spp. from dehydrated products.

Methods: Three different dehydrated matrixes were evaluated (Tomato, carrots and chicken broth). Samples were inoculated with *Salmonella,* three inoculum levels were used: high level (50-100 CFU/sample), low (1-5 CFU/sample) and non-inoculated samples. Ten samples were analyzed for each inoculum level and matrix combination. Twenty-five-g samples of the dehydrate matrices were added to 225 ml of buffered peptone water and incubated for 37°C/24 h. Samples were evaluated using the 3M Molecular Detection Assay (MDA). All samples were confirmed by the traditional method.

Results: One hundred percent of the inoculated samples were positive for MDA and the traditional method. Statistical difference between the two methods was not found ($P>0.05$). Sensitivity was 100% for the three matrices analyzed with the MDA. Negative controls yielded negative results. False negative are not reported.

Significance: 3M MDA can detect *Salmonella* present in dehydrated products providing next day results that are equivalent to those obtained by the traditional methods. The dehydrated matrices were not inhibitory to bacterial growth.

P3-85 Comparison of New and Traditional Culture-dependent Media for Enumerating Foodborne Yeasts and Molds

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Introduction: The 3M Petrifilm Rapid Yeast and Mold (RYM) Count Plate has been reported to compare favorably with traditional culture-dependent media for obtaining actionable yeast and mold counts in foods within incubation times of 48 to 60 h. Many of these foods were artificially inoculated.

Purpose: The purpose of this study was to compare RYM Petrifilm with other culture-dependent media for enumerating yeasts and molds in naturally contaminated foods.

Methods: Homogenates of 56 foods (6 dairy products, 5 meat and meat products, 15 fruits and fruit products, 5 vegetables, 9 cereal products, 8 seeds and nuts, and 8 dry seasonings and tea) were plated on RYM Petrifilm, YM Petrifilm, Dichloran Rose Bengal Chloramphenicol (DRBC) agar, Acidified Potato Dextrose Agar (APDA), and Dichloran 18% Glycerol (DG18) agar. Colonies were counted after incubating plates for 48, 72, and 120 h at 25°C.

Results: Out of 56 foods in which yeasts and/or molds were detected on one or more media (RYM, YM, DRBC, APDA, DG18) incubated for 120 h, neither yeasts nor molds were detected in 55.4, 73.2, 21.4, 19.6, and 71.4% and 3.6, 1.8, 0, 0, and 0% of foods plated on the five respective media and incubated for 48 and 120 h, respectively. The general orders of performance were DRBC = APDA > RYM Petrifilm > YM Petrifilm ≥ DG18 when plates were incubated for 48 h, DRBC > APDA > RYM Petrifilm > YM Petrifilm ≥ DG18 at 72 h, and DRBC > APDA > RYM Petrifilm = YM Petrifilm > DG18 at 120 h.

Significance: The incubation time at which actionable levels of yeasts and molds naturally present in a foods can be determined will depend on the origin, composition, pH, and a_w of the food being examined, the formulation of the enumeration medium, and standards set by the manufacturer and regulatory agencies.

P3-86 Simultaneous Detection and Prevalence of Allergens in *Anisakis* Species Isolated from Sea Fish

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Introduction: *Anisakis simplex* was first reported in the Netherlands in 1960 and over 20,000 cases of anisakiasis have been reported worldwide. In Korea, 107 cases of human anisakiasis to cause acute abdominal pain were reported. Since the allergenic potential of *A. simplex* was first addressed in Japanese urticaria patients who consumed mackerel, urticaria was known as a common allergic symptom in *Anisakis* allergy cases. While most of the previous studies have focused on the identification and characterization of individual Ani s allergens in *A. simplex* larvae, the prevalence and profiles of Ani s allergens have not yet been examined so far in anisakid larvae.

Purpose: This study aimed to develop a multiplex reverse transcription polymerase chain reaction (RT-PCR) for the detection of *Anisakis* allergens and to investigate the relationship of allergen profiles and anisakid larvae isolated from *Scomber japonicus*, *Trichiurus lepturus*, and *Conger myriaster* in Korea.

Methods: The visceral organs of 49 mackerel (*Scomber japonicus*), 45 large head hairtail (*Trichiurus lepturus*), and 46 whitespotted conger (*Conger myriaster*) were collected as samples from seafood markets and retailers. The *Anisakis* species was determined by *Anisakis pegreffii*-specific PCR and polymerase chain reaction-restriction fragment length polymorphism analysis. The prevalence and profile of 5 Ani s allergens were examined by multiplex RT-PCR.

Results: Among 140 larvae, *A. pegreffii* and *A. typica* accounted for 97.1% and 2.9%, respectively. The prevalence of each allergen in *A. pegreffii* was 41.2% for Ani s 1, 72.1% for Ani s 2, 69.9% for Ani s 3, 86.7% for Ani s 4, 93.4% for Ani s 5. Most *A. pegreffii* larvae had multiple allergen profiles, and 80.7% of *A. pegreffii* carried both Ani s 4 and Ani s 5 that are heat-resistant allergens. Especially, 52~65% of *A. pegreffii* isolated from *S. japonicus* and *C. myriaster* carried all 5 Ani s allergens.

Significance: This study clearly demonstrated the prevalence and profiles of Ani s allergens that have allergenic potential in *A. pegreffii and A. typica* larvae.

P3-87 *Cryptosporidium* Species and *Cyclospora cayetanensis* Surveillance in Fresh Produce and Herbs in Iowa

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Introduction: *Cryptosporidium* and *Cyclospora cayetanensis* are parasitic protozoans that cause gastrointestinal illness in humans. The parasites can be transmitted by food that has fecal contamination from an infected host. In 2013, there was a large outbreak of cyclosporiasis in Iowa (and other states) that was traced to lettuce. There was also a large increase in *Cryptosporidium* positive stool samples during this same time frame.

Purpose: In response, a food safety surveillance program was initiated by State Hygienic Laboratory and Iowa Department of Inspection and Appeals to test fresh produce (retail and farmers markets) for both parasites.

Methods: Two real-time polymerase chain reaction (qPCR) assays were developed for the rapid and sensitive detection of *Cryptosporidium parvum/hominis* and *Cyclospora cayetanensis* in leafy green produce. The *Cyclospora cayetanensis* PCR targets the 18S rRNA gene. The *C. parvum/hominis* assay is a multiplex PCR assay that amplifies a *Cryptosporidium* specific gene. The performance of the PCR assays on leafy green produce was evaluated by inoculation with oocysts and processed according to protocol. The methods are sensitive and able to detect *C. cayentanensis* and *C. parvum/hominis* parasites in leafy green products inoculated with 60 and 40 oocysts 100% of the time, respectively.

Results: Surveillance testing for *C. cayetanensis* was conducted for 105 packaged leafy green products from 15 different manufactures that were obtained from 13 Iowa grocery stores. *C. parvum/hominis* was tested for in 98 locally grown leafy green products from 37 different vendors/farms obtained from five different farmers markets. No parasites were detected in the 203 samples.

Significance: The 2013 *Cyclospora* outbreak suggested that the incidence of these parasites in produce may be of greater significance than previously recognized and sensitive real-time assays were needed. Surveillance testing will continue in 2016.

P3-88 Nanobiosensors for Foodborne Threat Detection

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❖ ***Developing Scientist Competitor***

Introduction: According to the United States Centers for Disease Control and Prevention (CDC) report, 48 million Americans get sick, 128,000 get hospitalized and 3,000 die each year due to foodborne pathogens. Several attempts have been made to detect those pathogens with high sensitivity and selectivity. The most routinely used methods include Enzyme-Linked ImmunoSorbent Assay (ELISA) and Polymerase Chain Reaction (PCR)-based

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assays. While ELISA has poor detection limit, PCR techniques are time-consuming and require trained personnel. In this work, we introduce a new ultrasensitive apta-assay based on liposome-mediated amplification of gold nanoparticle aggregation.

Purpose: The purpose of this work is provide an ultrasensitive assay capable of detecting very low levels of a target organism or agent with the naked eye.

Methods: We have specifically employed this assay using magnetic nanoparticle. In this assay, magnetic nanoparticles labeled with specified DNA receptors known as aptamers are used to capture pathogens in solution. Once the target pathogen is captured, cysteine-loaded liposomes (also labelled with the aptamer) are added to the system and are mixed with plasmonic gold nanoparticles. The liposomes then break down and release cysteine molecules, which in turn causes the aggregation of the gold nanoparticles and a color shift of the solution from red to blue purple.

Results: Our approach showed a detection limit below the zeptomolar level, and the ability to detect a single pathogen with the naked eye. Additionally, this method saves time by overcoming the need of laborious washing steps required by ELISA.

Significance: The assay reported here is a promising alternative for rapid and ultrasensitive detection kits of foodborne pathogens. This assay can also be applied to many other areas of food safety, protection and defense by simply changing the biological receptor (aptamers, antibodies, etc.) in order to select a different target agent.

P3-89 Determination of Penicillin G in Heavy Sow Urine Using Immunochromatographic Assay and Microbial Inhibition Swab Tests

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Introduction: Penicillin is a commonly used antibiotic in food animals. Unfortunately, violative penicillin residues in animal carcasses are sometimes identified by the USDA Food Safety and Inspection Service. Antemortem matrices such as urine could prove valuable for predicting possible violative tissue residues thus avoiding unnecessary carcass condemnation.

Purpose: To evaluate an immunochromatographic assay and kidney inhibition swab (KIS) test for the determination of penicillin G residues in sow urine.

Methods: Sows (n=126; 228±30.1 kg) were administered daily IM doses of penicillin G procaine (33,000 U/kg bw; 5x the label dose) for 3 consecutive days using 3 different administration patterns. Urine was screened for penicillin G using the KIS test as well as by an immunochromatographic assay. The urine samples were diluted with milk prior to processing for the immunochromatographic procedures. For the KIS test the presence of penicillin G was indicated by the development of a purple color.

Results: Using a simple modification consisting of the addition of whole milk, we were able to adapt a commercially available immunochromatographic assay designed for use in milk for use in identifying penicillin G in sow urine. Immunochromatographic assay results were comparable with the KIS test results with 96% accuracy. When KIS test accuracy was compared between assays performed in 2012 and 2015 there was 98% agreement, indicating stability of penicillin G in urine under storage conditions of -80°C. LC-MS/MS results confirmed penicillin G concentrations did not differ between measurement years.

Significance: Both KIS test and immunochromatographic assay methods can be performed "onsite" and produced similar results, but immunochromatographic assays provided "real time" (5 min testing time) answers in comparison to KIS test which require ~4 hours. Nevertheless, either rapid screening method could be used onsite to determine penicillin G residues in sow urine prior to marketing.

P3-90 A Novel Enzymatic Treatment to Remove Contaminating Free DNA in Phage-Treated Samples for Use in Routine Testing

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***Introduction*:** A common hurdle of PCR for detecting living bacteria in food lies in amplification of contaminating DNA that may originate from food processing, such as bacteriophage spraying for sanitization purpose. To date, only low-throughput protocols involving reactive and hazardous chemicals are available to get rid of such DNA that may interfere with the detection of living pathogens.

***Purpose*:** The purpose of this study was to evaluate an enzymatic approach to remove free *Listeria* DNA present in food samples treated with commercial phage suspension without affecting the PCR detection of living *Listeria*cells.

***Methods*:** Dilutions of living *L. monocytogenes* bacteria were spiked into 25g of Cheddar cheese portions that were treated or not with *Listeria* phage suspension. Samples were enriched for 24h in 225 mL of *Listeria* Special Broth. 100 µL aliquots were submitted to an enzymatic treatment and *Listeria* DNA was subsequently extracted with a specific lysis buffer and PCR amplified with the iQ-Check® *Listeria* spp. assay. Cq values for both *Listeria* targets and Internal Control were compared in presence or absence of the free DNA removal treatment.

***Results*:** Treating Cheddar cheese with *Listeria* phage led to a positive PCR signal with Cq in the 35-37 range, corresponding to approximately $5x10^4$ bacterial genomes per gram of cheese. This signal hindered the detection of living bacteria. When the sample was pretreated with the enzyme mix, free DNA was readily suppressed whereas living cells were detectable even at the lowest amount. Furthermore, the Internal Control signal was not impacted confirming that the extraction step efficiently neutralized the enzymatic reagent.

***Significance*:** These results demonstrate that a straightforward enzymatic method is efficient to clean food samples treated with phage suspension from free DNA without affecting the detection of living cells. The use of this method can be easily extended to other food matrices that are prone to the presence of dead cells.

P3-91 Development and Validation of a Gluten-specific Sandwich ELISA based on a Novel Monoclonal Antibody, 2D4

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Introduction: Cereal grains contain gluten, consisting of prolamin and glutelins. The prolamin fraction from wheat and related *Triticeae* can be immunopathogenic. Consumption of these grains is associated with celiac disease (CD). Because CD manifests as debilitating symptoms and chronic complications, affected individuals must adopt a gluten-free diet. Avoiding gluten is challenging because foods labeled "gluten-free" are frequently contaminated with gluten. In response, regulatory authorities have established allowable gluten levels in foods labeled "gluten-free" to protect consumers from unanticipated exposures.

Purpose: We have generated a novel monoclonal antibody (mAb), 2D4, directed against the prolamins of *Triticeae* grains with improved characteristics relative to the R5 mAb.

Methods: A sandwich ELISA using 2D4 and denaturing buffer was validated using prolamin standards derived from wheat, barley, rye, oats, and deamidated wheat protein and 35 common commodities, for cross reactivity, interference, and LOD. We also characterized prolamin extractability from complex foods and resistance of the assay to temperature deviations.

Results: The 2D4-based sandwich ELISA uses a 20 min extraction period, and ELISA incubation steps of 10 min, 10 min, and 5 min. There was no cross-reactivity with any of the commodities tested. Spike analysis averaged 85% recovery. The LOD was established at 1 ppm prolamin or ~2 ppm gluten. Analysis of complex foods demonstrated improved recovery in thermally processed foods using the denaturing buffer system as compared to standard 60% ethanol extraction. Assessment of the assay at 18, 22, and 25°C showed resistance to temperature deviations.

Significance: The development of a highly sensitive and rapid test method capable of accurately detecting trace amounts of prolamin residues (modified and native) in under 45 min should aid food manufacturers and regulatory entities in monitoring for gluten and gluten derivatives that have previously proved challenging.

P3-92 Automated DNA Purification from Food for Authentication and Genetically Modified Organism (GMO) Testing

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Introduction: Determining whether foods are labeled correctly for species or GMO content is a major concern to the food industry. The ability to test samples using molecular technologies is useful but can be time consuming and some methods lead to user variation.

Purpose: Here, we report the utility of a small bench top, automated instrument, the Maxwell RSC and a new kit chemistry for purification of amplifiable DNA from a variety of foods and feed. We tested seeds for GMO and meat for species authentication.

Methods: Nucleic acid was extracted from 50-200 mg ground seeds or meat using the Maxwell RSC PureFood GMO and Authentication kit. For GMO testing, extracted DNA from corn, soybean and canola was tested for the presence of cauliflower mosaic virus (CaMV) or Figwort Mosaic Virus (FMV) promoter and a plant-specific sequence. For seafood authentication, DNA was extracted from fish and amplified using endpoint PCR of cytochrome *b*, followed by restriction enzyme digestion and RFLP analysis. Ground beef and pork testing focused on isolation of nucleic acid from low level mixtures of pork in beef with analysis by quantitative PCR of pork- or beef-specific mitochondrial DNA sequences.

Results: The seed GMO testing showed amplification of virus promoters in GMO labeled samples and no amplification or only trace amounts in the non-GMO samples. The fish samples were identified as correctly matching the species listed on labels. The mixed pork and beef sample amplification results correlated to the percentage of the mixture. DNA purification of the same sample in 3 different experiments was reproducible (n=8 each run, CVs < 13%). There was no detectable cross-contamination and no inhibition of amplification in the eluates.

Significance: These studies together demonstrate the utility of the Maxwell RSC System for automated purification of food DNA upstream of amplification-based GMO and authentication testing.

P3-93 Evaluation of Human Norovirus Transmission with Virus-Like Particles

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Introduction: Human noroviruses (HuNoVs) cause acute gastroenteritis worldwide. However, whether disinfections particularly in living environments, can effectively prevent infections by HuNoVs has been unclarified because the quantitative evaluation of HuNoV transmission has been limited.

Purpose: The aims of this study were to develop a method to evaluate HuNoV transmission quantitatively using a surrogate agent and to clarify their transmission on the basis of activities of daily living.

Methods: Virus-like particles (VLPs) of the HuNoV TCH strain used as the surrogate agent were produced using a baculovirus expression system. Stainless steel plates contaminated with 4 ng of VLPs (corresponding to 2.2×10^8 of HuNoV particles) were wiped with a piece of paper or cotton cloth wet with sterilized water, which was then used to wipe other new stainless steel plates. The VLPs were collected from each stainless steel plate and quantified by ELISA (n = 4).

Results: The amounts of VLPs remaining on the stainless steel plates after the first wiping with the piece of wet paper or cotton cloth were 0.39±0.18 and 0.46±0.14 ng, respectively. The amounts of VLPs transmitted to the new stainless steel plates after spreading with the same piece of paper or cloth were 0.070±0.034 and 0.34±0.050 ng, respectively, both of which corresponded to more than 10^2 HuNoV particles, which is the number of particles sufficient to cause infection.

Significance: The evaluation method for HuNoV transmission using VLPs could be effective from the viewpoint of sanitization. The results suggest that wiping could easily transmit sufficient amounts of HuNoVs for infection and cannot remove them completely from the surfaces of materials in living environments.

P3-94 A Semi-mechanistic Modeling Approach to Describe the Transfer of *Listeria monocytogenes* during Slicing of Ready-to-Eat Cooked Ham

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Introduction: Processed meat products can become contaminated with *Listeria monocytogenes* due to cross-contamination during post-processing procedures performed outside the industrial environment, such as slicing at retail or food services. Models able to describe and explain the transfer are essential to assist food processors and authorities to access risks and to improve food safety.

Purpose: The study aimed to apply a modeling approach able to describe and explain the transfer of *L. monocytogenes* during slicing of cooked ham at retail, based on data generated in a laboratorial setting, in which a manual slicer was experimentally contaminated with the pathogen.

Methods: The slicer was contaminated by slicing a cooked ham piece that has been immersed in a suspension containing *L. monocytogenes* (8 log CFU/ml). Subsequently, non-inoculated cooked ham was sliced in this slicer, until 190 slices were obtained. This setup was performed nine times, testing different levels of inoculation and blades. Counts of *L. monocytogenes* were done using a standard method. The results were fitted to existing cross contamination models and a new model was suggested.

Results: The suggested model hypothesized that the input of *Listeria* is organized in two different environments; one where *Listeria* transfer ability from the slicer to the ham is high and a second where the transfer is much lower. The model is described by a four-parameter equation Log (L_{i+k})= $\log[b_1(1-b_1)^{k-1}E_{1,i} + b_2(1-b_2)^{k-1}E_{2,i}]$, where E_1 and E_2 represent the levels of *L. monocytogenes* in the two environments after slicing the contaminated ham. The proposed model presented low RMSE values varying between 0.02 – 0.06.

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Significance: The proposed model was able to describe the transfer *of L. monocytogenes* during slicing of ready-to-eat cooked ham. This model worked with fewer parameters and presented the best fit among the studied models.

P3-95 Integrated Multiphysics-microbial Kinetics Model for Predicting Heating and Microbial Inactivation Performance during Microwaving Mashed Potato

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❖ ***Developing Scientist Competitor***

Introduction: Nonuniform heating is the biggest issue of microwaveable food. The pathogens in the cold spots may not be completely inactivated after heating, which may cause severe food bore illness.

Purpose: Prediction of microbial inactivation during microwave heating process will be useful to evaluate the safety of microwaved food products.

Methods: In this study, microbial thermal inactivation equation was integrated with multiphysics (electromagnetics and heat transfer) equations of microwave heating in a 3-dimensional finite element model to predict the heating and microbial inactivation performance during microwave cooking of mashed potato. The mashed potato (550 g) was cooked on a rotating turntable in a 1200 W domestic oven for four minutes. The efficiency of inactivation of *Escherichia coli* K12 (*E. coli*) in mashed potato was evaluated during the cooking (four minutes) and standing times (four minutes) in the model.

Results: The results showed that nonuniform temperature distribution in the mashed potato caused uneven microbial inactivation during cooking. The microbial distribution profile matched closely with the temperature profile. The coldest spot (49.6°C) was found at the center of the mashed potato with 0 log reduction of *E. coli* after four minutes of microwave heating. After four minutes of standing time, the coldest spot in the mashed potato achieved 5.4 log reduction of *E. coli*, showing that standing time after microwave heating is very important for improving the safety of the microwaveable food products.

Significance: The coupled model can be used by the food developers to assist designs of microwaveable food products that deliver more uniform heating and safety.

P3-96 Kinetics and Thermodynamics of Thermal Inactivation of Novel Bacteriophages Specifically Targeting Non-O157 Shiga-toxigenic *Escherichia coli*

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❖ ***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 as bio-preservatives in ready-to-eat meat and poultry products has paved way for development of phage-based antimicrobials. Understanding their thermal inactivation kinetics is essential to allow their use as bio-preservatives in food matrices. Kinetic modeling of thermal inactivation of STEC-specific bacteriophages could enable their predictive survival for successful application in food systems.

Purpose: Evaluation of thermal inactivation models for selected bacteriophages and analysis of thermodynamic parameters to understand denaturation.

Methods: Bacteriophages previously isolated from cattle farms in Oklahoma and those exhibiting inhibition towards non-O157 STEC (O26, O45, O103, O111, O121, and O145) were used. Thermal inactivation of bacteriophages (at 8-9 log PFU/ml), was determined at 40-90°C for 60 minutes with 10-minute-sampling intervals. Phages were heated in sealed tubes with host STEC-strain and enumerated using double-agar-layer technique. Kinetic degradation models from published literature: first-order, two-fraction, Weibull and n^{th}-order were used to understand degradation kinetics through non-linear regression module, using SigmaPlot13 (Systat Software, Inc., CA, USA). Activation enthalpy (ΔH), free energy of inactivation (ΔG) and activation entropy (ΔS) was calculated using rate-constant and activation energy (*Ea*) values.

Results: The n^{th}-order-model provided the best description of thermal inactivation of bacteriophages at selected temperatures. The r^2 values ranged from 0.74-0.91 for all phages. High *Ea* values ranging from 195-619 kJ mol^{-1} indicate that bacteriophages are relatively more thermostable than bacteriocins. *D*-values at reference temperature (t_{ref}) ranged from 22-346 minutes. High positive values of ΔH and ΔS was observed in our results. Thermodynamic parameters revealed that tested bacteriophages had structural thermo-stability.

Significance: Bacteriophages could serve as biopreservatives in food industry to provide synergistic effect with heat treatment.

P3-97 Development of Predictive Model for *Campylobacter jejuni* Survival on Beef Tartare

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❖ ***Developing Scientist Competitor***

Introduction: Recently, foodborne illness caused by *Campylobacter* spp. has been increased in S. Korea, especially by raw meat. Beef tartare is a food eaten raw, and this is consumed commonly in certain countries. For this reason, the probability of *Campylobacter* foodborne illness could be high.

Purpose: This study developed the models to predict the survival of *Campylobacter jejuni* in beef tartare.

Methods: A mixture of *C. jejuni* strains ATCC33560 and NCTC11168 was prepared, and 0.1 ml of the mixture were inoculated into beef tartar samples (25 g) to obtain 6-7 log CFU/g, followed by aerobic-storage at 4°C, 10°C, 15°C, 25°C, and 30°C for up to 984 h, depending on temperature. During storage, samples were analyzed microbiologically to enumerate *C. jejuni* populations with modified CCDA-Preston. The survival data were analyzed with the Weibull model to calculate *δ* which is required time (h) for first decimal reduction and *p* which describes the shape of curves. The parameters were then further analyzed with the Davey model to describe the effect of temperature on the parameters. Subsequently, root mean square error (*RMSE*) was calculated to evaluate the differences between observed and predicted data from models.

Results: *C. jejuni* cell counts were gradually decreased at low temperatures (4°C and 10°C), but the cell counts were dramatically decreased ($P<0.05$) at 15°C, 25°C, and 30°C. *δ* values decreased from 657.1 h to 9.7 h as storage temperature increased, and temperature effect on curve shape was observed. In addition, the secondary model with the Davey model was appropriate with 0.927 and 0.731 of R^2 in *δ* and *p*, respectively. From validation, *RMSE* with 0.475 suggested that prediction by the developed model was appropriate.

Significance: These results indicate that *C. jejuni* can survive longer at lower temperature, and the developed models should be useful in predicting *C. jejuni* survival in beef tartare.

P3-98 Mathematical Model to Describe the Fates of *Campylobacter jejuni* on Raw Beef Liver

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Introduction: *Campylobacter* spp. have been isolated from fresh meats such as poultry and beef, and usually cause campylobacteriosis by cross-contamination. However, if the products are eaten raw, the probability of the foodborne illness could be increased, especially for raw beef offal such as liver in certain countries.

Purpose: The objective of this study was to evaluate the fate of *Campylobacter jejuni* in raw beef liver with predictive models.

Methods: Raw beef liver was purchased from a wet market in S. Korea, and cut into 25-g portions. A mixture (0.1 ml) of *C. jejuni* strains ATCC33560 and NCTC11168 was inoculated into samples to obtain 6-7 log CFU/g, and the samples stored aerobically at 4°C, 10°C, 15°C, 25°C, and 30°C. *C. jejuni* cell counts in samples were then 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* (the shape of curves). *Delta* were further analyzed with a secondary model (Davey model) as a function of storage temperature. The predicted data were compared with observed data, and root mean square error (*RMSE*) was calculated to evaluate the accuracy of the model prediction.

Results: *C. jejuni* cell counts were not changed at 4°C during storage, but dramatically decreased ($P<0.05$) at 10°C, 15°C, 25°C, and 30°C. The *Delta* values from the primary model were 1,344 h for 4°C, 157.5 h for 10°C, 54.2 h for 15°C, 22.7 h for 25°C, and 2.2 h for 30°C. Also, no differences in *p* values among temperatures were observed. The secondary model well-described the temperature effect on *Delta* with 0.842 of R^2. *RMSE* with 0.859 suggested that the accuracy of model performance was acceptable.

Significance: This result indicates that the developed model should be useful in describing the fate of *C. jejuni* in raw beef liver.

P3-99 Quantitative Microbiological Risk Assessment of *Campylobacter* spp. on Raw Meat in Korea

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Introduction: *Campylobacter* spp. have been isolated from fresh poultry, beef, and pork. In some area, raw meats are consumed without cooking. Thus, there are high possibility for *Campylobacter* foodborne outbreaks through the product intake.

Purpose: The objective of this study was to assess of the risk of *Campylobacter* spp. in raw meat such as beef and chicken breast in S. Korea.

Methods: For hazard identification, the general characteristics and outbreaks for *Campylobacter* were reviewed. For exposure assessment, the prevalence of *Campylobacter* spp. in beef tartare (BT) and chicken sashimi (CS), and conditions for distribution and storage of the meat were surveyed, followed by @RISK fitting program. Also, predictive models of *Campylobacter* in raw meat, and consumption amount and frequency for the raw meats were investigated. Dose-response models for *Campylobacter* were searched though literatures. Eventually, simulation models with @RISK were developed using the collected data to estimate the risk of *Campylobacter* foodborne illness by the intake of BT and CS.

Results: There was difference in the prevalence between CS and BT samples. Predictive models developed with the Weibull, Polynomial and Davey models were cited to exposure assessment. Appropriate probabilistic distribution for consumption amount was exponential distribution with 66.7 g (CS) and 174.3 g (BT) of mean only for the people who eat these products. Consumption frequencies per month were 0.6% and 11.7% for CS and BT, respectively. Through simulation with the collected data, the risks of *Campylobacter* foodborne illness per day were 2.37×10^{-8}-4.14×10^{-4} and 3.90×10^{-6}-1.06×10^{-3} for CS and BT, respectively.

Significance: Although the risk of *Campylobacter* spp. in raw meat was low for entire populations in S. Korea, the risk was very high for the people who consume raw meat. Thus, a measure is necessary to reduce the risk of *Campylobacter* in raw meat.

P3-100 Survival of *Salmonella* on the Surface of Plastic Grocery Bags through Leakage from Raw Chicken Packages

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Introduction: Consumers often keep the grocery bags from shopping trips to reuse for other purposes. There are food safety concerns in reusing the bags that were used in wrapping raw poultry products. The leakages from the packages can often carry foodborne pathogens such as *Salmonella*. However, there is no scientific data to assess such risk. It is not clear how long *Salmonella* from the leakages can survive on the surface of plastic grocery bags.

Purpose: The objective of this study was to evaluate the attachment and continued existence of *Salmonella* from leakages onto plastic grocery bags.

Methods: *Salmonella* cells were mixed with the meat juices collected from chicken packages. The mixtures were applied to the surfaces of pieces of plastic bags cut into 2 in. x 2 in. squares either uncovered or covered with another piece of plastic on top. *Salmonella* on the surfaces were enumerated with both selective (XLT-4) and non-selective (APC) agar methods for two weeks and the experiments were repeated five times.

Results: In the covered condition, it took 4.2 and 8.8 days to reduce *Salmonella* counts to one-thousandth of the original numbers when enumerated with XLT-4 and APC, respectively. In the uncovered condition, it took 12.0 and 22.4 days to reach the same levels of *Salmonella* with XLT-4 and APC, respectively. The longer surviving days observed in the uncovered condition implied a protective mechanism involving the dryness, oxygen exposure, and protein film. The longer surviving days observed with the non-selective method indicated better recovery rates of *Salmonella* than that with the selective method. The harshness of selective agents may suppress the recovery of *Salmonella* from the surfaces.

Significance: *Salmonella* can persist on the plastic surface for long period of time. It is important not to reuse the bags that had been used in wrapping raw poultry packages to prevent cross contamination.

P3-101 A Dynamic Model to Predict the Fates of *Listeria monocytogenes* in Nappa Cabbage Kimchi under Changing Temperature

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❖ ***Developing Scientist Competitor***

Introduction: During kimchi fermentation, storage temperature can be one of the most important factors related to the growth of foodborne pathogens, and the temperature is usually changing rather than being constant.

Purpose: This study developed a dynamic model to predict the fates of *Listeria monocytogenes* in Nappa cabbage kimchi during fermentation under changing temperatures.

Methods: A mixture of *L. monocytogenes* strains NCCP10805, NCCP10806, NCCP10807, NCCP10808, NCCP10809, NCCP10810, NCCP10811, NCCP10920, and NCCP10943 was inoculated into Nappa cabbage kimchi at 4 log CFU/g. They were then incubated at 4°C (14 days), 15°C (2 days), and 25°C (1.5 days). *L. monocytogenes* and lactic acid bacteria cell counts were enumerated on PALCAM, and de Man, Rogosa and Sharpe agar, respectively. The microbiological data were fitted to the Baranyi model to estimate the kinetic parameters such as growth rate or death rate (log CFU/g/h), and lag phase duration (LPD; h) or shoulder period (h). The kinetic parameters were then fitted to a polynomial model. A dynamic model was eventually developed, and root mean square error (*RMSE*) was calculated to evaluate the model performance.

Results: *L. monocytogenes* growth was observed at 15°C and 25°C for cabbage kimchi, and the bacterial cell counts decreased (P<0.05) as lactic acid bacterial cell counts increased. Growth rate and death rate values from the Baranyi model were increased (P<0.05), but LPD or shoulder period were decreased (P<0.05) as storage temperature increased. A polynomial model then described well the effect of temperature on kinetic parameters, and a dynamic model was eventually developed to describe the fate of *L. monocytogenes* under changing temperature. *RMSE* suggested that the developed models had a good performance.

Significance: These results indicate that the developed models should be useful in predicting the fates of *L. monocytogenes* in Nappa cabbage kimchi fermentation at changing temperatures.

P3-102 Kinetic Behavior of *Listeria monocytogenes* in Diced Radish Kimchi

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Introduction: Although kimchi is known to become safe microbiologically through fermentation, foodborne outbreaks and recalls, especially for *Listeria monocytogenes*, have been occurred.

Purpose: The objective of this study was to develop a predictive model to describe the kinetic behavior of *L. monocytogenes* in diced radish kimchi.

Methods: A 9-strain mixture of *L. monocytogenes* (NCCP10805, NCCP10806, NCCP10807, NCCP10808, NCCP10809, NCCP10810, NCCP10811, NCCP10920, and NCCP10943) was inoculated in diced radish kimchi, and the target level was 4 log CFU/g. The samples were then stored at 4°C (14 days), 15°C (10 days), and 25°C (10 days), and *L. monocytogenes* (PALCAM) and lactic acid bacteria (de Man, Rogosa and Sharpe agar) were enumerated. *L. monocytogenes* cell count data were fitted to a primary model (Baranyi model) to calculate death rate (log CFU/g/h).

Results: In the primary model, the death rates of *L. monocytogenes* sin diced radish kimchi were decreased slightly as storage temperature increased, thus, the secondary model was not developed. However, the bacteria maintained survival at initial level during fermentation at all temperatures, indicating that the pathogen might not be affected by lactic acid bacteria.

Significance: This result indicates that *L. monocytogenes* may be a risk if they present initially in diced radish kimchi during fermentation.

P3-103 The Fates of *Salmonella* in Diced White Radish Kimchi under Changing Temperatures

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Introduction: Changing temperature and storage time are critical factors for the behavior of *Salmonella* in diced white radish kimchi.

Purpose: This study developed a dynamic model to predict the fates of *Salmonella* in diced white radish kimchi during fermentation.

Methods: Diced white radish kimchi was prepared in laboratory by a traditional method. A 5-strain mixture of *Salmonella* (Typhimurium NCCP10812, Agona NCCP12231, Enteritidis NCCP12243, *S. enterica* KACC11595, and Montevideo NCCP10141) was inoculated in diced white radish kimchi at 4 log CFU/g. The inoculated samples were then stored at 4°C, 15°C, and 25°C for 14 days, 2 days, and 1.5 days, respectively. *Salmonella* (XLD) and lactic acid bacteria (de Man, Rogosa, and Sharpe agar) was enumerated, and the observed data of *Salmonella* were fitted to the Baranyi model to calculate death rate (*DR*; log CFU/g/h) and shoulder period (h). The kinetic parameters (*DR* and shoulder period) were then fitted to polynomial equations, and a dynamic model was developed in accordance with primary and secondary models. To validate the performance of developed models, root mean square error (*RMSE*) was calculated.

Results: *Salmonella* did not grow at all temperatures, and the death of *Salmonella* started after shoulder periods as bacterial populations of lactic acid bacteria increased. From 4 to 25°C, the shoulder period was rapidly decreased (P<0.05), and *DR* increased (P<0.05). Developed secondary models for the kinetic parameters showed appropriate performance. As a result of validation, the performance of kinetic models was appropriate with 0.485 of *RMSE*. The prediction of developed dynamic model at changing temperatures was also appropriate.

Significance: These results indicate that the developed models should be useful in predicting the kinetic behavior of *Salmonella* in diced white radish kimchi fermentation.

Poster

P3-104 Mathematical Models to Predict the Behavior of *Salmonella* in Napa Cabbage Kimchi under Dynamic Temperature

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Introduction: Kimchi is traditional food in South Korea, and most Koreans consume kimchi with main dish. Although foodborne illness by *Salmonella* in kimchi has not occurred, the pathogen can cause outbreak due to the presence of the pathogen in ingredients of Napa cabbage kimchi.

Purpose: In this study, kinetic models and a dynamic model were developed to predict the kinetic behavior of *Salmonella* in Napa cabbage kimchi under changing temperature.

Methods: A mixture of *Salmonella* (*Salmonella* Typhimurium NCCP10812, *Salmonella* Agona NCCP12231, *Salmonella* Enteritidis NCCP12243 *Salmonella enterica* KACC11595, and *Salmonella* Montevideo NCCP10141) was inoculated to Napa cabbage kimchi at 4 log CFU/g. The samples were then stored at 4°C (16 days), 10°C (10 days), 15°C (6 days), 25°C (3.5 days), and 30°C (2 days). *Salmonella* and lactic acid bacterial cell counts were enumerated on xylose lysine deoxycholate agar, and de Man, Rogosa and Sharpe agar, respectively. The kinetic parameters such as growth rate or death rate (log CFU/g/h), and lag phase duration or shoulder period (h) were calculated by the Baranyi model, and they were then fitted to a polynomial model. A dynamic model was developed, and root mean square error (*RMSE*) was estimated to validate model performance.

Results: Lactic acid bacterial growth influenced *Salmonella* growth during fermentation. Growth rate and death rate values calculated by the Baranyi model were increased (P<0.05), but lag phase duration and shoulder period were decreased (P<0.05) when storage temperature increased. A polynomial model was appropriate to describe the effect of temperature on the kinetic parameters, and developed dynamic model was also appropriate to describe the fate of *Salmonella* under changing temperature. *RMSE* indicated that the developed models had a good performance.

Significance: These results indicate that the developed models should be useful in predicting the kinetic behavior of *Salmonella* in Napa cabbage kimchi at changing temperatures.

P3-105 Building Better Microbial Growth Models: Estimating the Influence of Nutrient Diffusion Rate on the Transition Period from Exponential to Stationary Phase Using *Escherichia coli* k-12

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❖ ***Developing Scientist Competitor***

Introduction: One of the long term goals in modeling microbial growth in foods is development of more mechanistically-based models. Currently, most models are based on studies in liquid culture where growth kinetics are largely independent of inoculum size. However, in solid matrices, it can be hypothesized that the initial inoculum size would affect nutrient diffusion rates during late exponential and early stationary phase, thus affecting the shape of growth curve.

Purpose: The purpose of the study was to evaluate if inoculum size influences the growth kinetics of *Escherichia coli* K-12 in a solid matrix.

Methods: *E.coli* K-12 cells were grown in BHI broth to early stationary phase and then diluted to obtain the desired inoculum sizes (from 10^2 to 10^6 CFU/ml). The inocula were transferred to 2% (wv/vol) agar system, solidified, and then overlaid with additional top agar to prevent surface growth. The cultures were incubated at 37°C and sampled for designated time period until early stationary phase. Viable counts data were fitted using Baranyi model (IPMP 2013).

Results: The movements of *E.coli* K-12 cells were restricted resulting in the formation of micro-colonies in the agar matrix. With lower inoculum sizes, the longer transport distance resulted in limiting nutrient diffusion during late exponential phase. The transition period from exponential to stationary phase was influenced strongly by inoculum size, with higher inoculum sizes leading to a more abrupt transition, while lower inoculum sizes lead to more gradual attainment of maximum population densities.

Significance: The results show the possibility of developing more effective growth models that better depict the growth of bacteria in solid food systems. These results are an important step in developing more mechanistically-based food safety and food quality models that take into consideration of phase-dependent physiological events.

P3-106 Evaluation of Growth and Metabolic Variations of *Salmonella* spp. Strains Related to Host-specificity Using Computational Metabolic Models

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Introduction: To develop strategies fighting S*almonella* infections we need to understand the pathogen's capability on metabolizing achievable nutrients within specific hosts or niches during infection. Genome-scale metabolic models (GEMs) are useful for interpreting complex bacterial metabolic systems to generate metabolic predictions used during host-interactions.

Purpose: The objective of this project was to construct GEMs for five *Salmonella* strains and to analyze differentiating metabolic capabilities through model predictions.

Methods: *Salmonella* Abaetetuba str. ATCC 35640, *Salmonella* Enteritidis str. P125109, *S.* 4,[5],12:i:- str. CVM23701, *Salmonella* Typhimurium str. LT2 and str. UK1 were chosen for analysis. The semi-automated resource KBase was used to generate draft GEMs. In silico nutrient utilization predictions for individual carbon sources under aerobic and anaerobic conditions were conducted, and compared to in vitro data, leading to refinement and improvement in the GEMs predictive accuracy.

Results: Each GEM contained >1,250 metabolites participating in >1,330 reactions, and 1252 reactions are shared in common for all strains. There are 67 reactions unique to the GEM of str. LT2, 13 unique to the GEM of str. CVM23701 and 2 unique to the GEM of str. UK1 compared to the rest. Experimental data of str. LT2, str. UK1 and str. ATCC 35640 showed that from the 95 carbon sources tested in vitro, 72 were present in all three GEMs thus allowing validation of the models predictive accuracy, and the agreements between in silico and in vitro results were >90% for carbon utilization thus permitting simulation of multi-nutrient host niches for all *Salmonella* strains.

Significance: Although there are multiple GEMs built for foodborne pathogens, prior to this study there was only one GEM for a *Salmonella* strain. The high predictive accuracy for these *Salmonella* GEMs represents new tools for detection, control and prevention of *Salmonella* spp. in food and in hosts.

Poster

P3-107 Predictive Models of Behavior of *Staphylococcus aureus* for the Quantitative Microbial Risk Assessment in Processed Meat Products in Korea

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Introduction: Foodborne outbreaks related to processed meat products have been increasing over the world. In Korea, the Westernization of the Korean diet is driving the increase in the meat consumption. According to the increasing meat consumption, the safety of meat products has become more important.

Purpose: This research aims to develop a predictive model of behavior of *Staphylococcus aureus* for the quantitative microbial risk assessment and to establish a scenario including consumption amount, frequency and dose response model for simulation.

Methods: For developing growth prediction models, five processed meat products, gamja-tang, galbi-tang, samgye-tang, gom-tang, and pyeonyuk, were inoculated with the initial level of 103 CFU/g and the growth was monitored at different time and temperature points to establish the primary and secondary predictive growth model. More than 1,600 processed meat samples were collected from all region of Korea and tested for the contamination of *S. aureus*.

Results: Among 1,695 processed meat products tested for microbial contamination, only three samples were confirmed *S. aureus* contamination. A model for microbial growth prediction in processed meat products was successfully developed based on the renowned "Baranyi model" and "Square root model." As a result of simulation, the possibility of foodborne outbreak caused by *S. aureus* in processed meats was very low, suggesting the risk level of *S. aureus* in processed meats was very low under current zero-tolerant "Korea Food Code Standard." The most prominent factors that affect to the simulation result were types of meat products and frequency of intake, followed by initial contamination level, storing temperature.

Significance: This research are to review the possibility of establishing scientific standards and seek the advancement in the production of domestic food products by conducting a quantitative microbial risk assessment in processed meat products of which the consumption has been rapidly increasing.

P3-108 Modelling Growth of Single Cells and Cell Populations from *Pseudomonas aeruginosa*

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Introduction: *Pseudomonas aeruginosa* is not only a dominant spoilage bacterium in pork products but also an opportunistic human pathogen which could cause diarrhea and other diseases. Instead of a determined growth trend predicted by traditional microbiology methods, a stochastic growth prediction of *P. aeruginosa* single cells was needed to portray by taking into account heterogeneity of bacterial single cells.

Purpose: This study was designed to model the growth of single cells and cell populations from *P. aeruginosa*, and two factors, inoculum size and temperature shift, were considered.

Methods: A single cell growth image system was used to study the growth of *P. aeruginosa* single cells. A stochastic growth simulation method was developed to connect the growth of *P. aeruginosa* single cells and cell populations. And the lag time distributions with different inoculum sizes were obtained as a result of the simulation's repetitive executions. In addition, the growth of *P. aeruginosa* single cells under different temperature shifts was investigated.

Results: The lag time decreased as the inoculum sizes increased. Integrated by an Individual-based Modelling (IbM) process, the parameters of the growth of *P. aeruginosa* single cells were then introduced into the reduced Baranyi & Roberts model to fit for stochastic growth simulations of *P. aeruginosa*. Moreover, the growth of *P. aeruginosa* showed a determinate tendency as the inoculum sizes increased. The stochastic growth of *P. aeruginosa* single cell under different temperature shifts was simulated using an IbM process, and it showed that an aggravated variability of the growth of *P. aeruginosa* was more affected by bigger temperature shifts.

Significance: It was deduced that studying the microbial dynamics at the single cell level, which take into account the growth viability and uncertainty of bacterial single cells, could help to establish a more reliable set of microbe-influenced food shelf life and food safety criteria.

P3-109 Development of User Friendly Software Named KATS for Microbial Risk Assessment

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Introduction: In recent, microbial risk assessment (MRA) has been conducted in many countries to improve food safety, but people who is able to conduct the assessment are limited because of the complexity of the procedures for microbiological risk assessment. Hence, a user friendly software for MRA needs to be developed.

Purpose: The objective of this study was to develop a user friendly MRA software named KATS (Korean Analytical Tool for Safety).

Methods: To construct the database of KATS, the accuracy of all research reports for prevalence data of foodborne pathogens and predictive models in S. Korea were validated through simulation with @RISK. Data for distribution and storage conditions, cooking conditions, consumption patterns, and dose response models were collected from literatures. These data were then loaded into data base. In addition, Monte Carlo simulation and various probabilistic distributions were loaded in KATS. KATS system was programed with C# language for Windows. The performance of KATS was evaluated by comparing the results from @RISK.

Results: Of validated prevalence of foodborne pathogens and predictive models, about 10% of the data was determined to be loaded in KATS database. KATS system was organized into the flow of exposure assessment (selection of food-foodborne pathogen, initial contamination, distribution and storage conditions, cooking conditions, and consumption pattern), hazard characterization (dose response model), and risk characterization. Simulation results by KATS were in the accordance of 99.99% of @RISK.

Significance: This result indicate that KATS can be used as a user friendly software for MRA, and thus, the time and cost required for MRA can be minimized.

P3-110 Down-weighting Older Outbreaks in Estimates of Foodborne Illness Source Attribution

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Introduction: Outbreak data are used to estimate the proportion of foodborne illnesses due to food categories. Recent outbreaks may best reflect current risks, but excluding older outbreaks leads to data sparseness challenges and potential biases, particularly for foods less frequently identified as sources in outbreaks. This study was undertaken by the Interagency Food Safety Analytics Collaboration.

Purpose: We evaluated multiple approaches to down-weighting older outbreak data used to estimate foodborne illness source attribution. Whereas there is no objective notion of a 'median' down-weighting, we sought a robust compromise between using all data and using only recent data.

Methods: We identified 952 outbreaks in CDC's Foodborne Disease Outbreak Surveillance System (FDOSS) caused by four priority pathogens (*Salmonella*, *Escherichia coli* O157, *Campylobacter*, and *Listeria monocytogenes*) from 1998-2012 in which a food was implicated. We explored an array of approaches for down-weighting older outbreaks in a model for estimating attribution fractions.

Results: Excluding older outbreaks resulted in estimates of no attributable risk for some food categories. Including older outbreaks without applying down-weighting resulted in higher estimated attributions for some food categories with markedly decreased outbreaks over time. We chose a compromise from the array of down-weighting schemes based on an ad hoc 50% minimum weight for the most recent 5 years and 10% maximum for older information: down-weight data from outbreaks older than 5 years using an exponential decay function of 0.71. Using this approach, 67% of the information used to estimate attribution came from 2008-2012, 28% from 2003-2007, and 5% from 1998-2002.

Significance: This study identified an approach to limiting the influence of older outbreaks in attribution estimates without losing valuable information for pathogens and food categories with sparse data. Improved attribution estimates can provide essential information to regulators and public health officials for targeting risk interventions.

P3-111 Open-source Software for Foodborne Disease Outbreak Investigations Integrating Food Sales Data

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Introduction: Contaminations in food products can pose a significant threat to human or animal health. Despite significant improvements in analytical methods and surveillance systems it is still not possible to completely prevent disease outbreaks caused by contaminated feed or food products.

Purpose: We are developing a new software module for the open-source software platform FoodRiskLabs, which is designed to support risk assessors and decision makers in regular risk assessments and outbreak investigations. Specifically, the new software components support the identification of a disease-causing "contaminated" product by comparing the products sales distribution pattern with the spatial distribution pattern of human infections.

Methods: The new software module extends the collection of FoodRiskLabs tools which are based on the open-source data analytics platform KNIME. It includes the likelihood-based approach introduced by Kaufman et al. (2014), and provides support for parallelized or cloud-based execution. Further, a new Monte Carlo simulation-based algorithm has been developed that allows to identify the minimal set of products containing the "guilty" product within a user-defined confidence limit.

Results: The software features were tested on artificial outbreak scenarios generated from real world sales data. It was used to study the performance effects of three influencing factors using the extended likelihood-based approach. These analyses confirmed that for the given scenario settings the number of products under suspicion and the spatial granularity of the available data strongly influence the algorithm's performance.

Significance: The new FoodRiskLabs extension will be made freely available for download and joint development as an open-source community resource (https://foodrisklabs.bfr.bund.de). It provides a scalable software infrastructure enabling food authorities (and private sector stakeholders) to include food sales data into their outbreak investigations. Further research is necessary to address remaining open questions with respect to the algorithm's performance in cases where the underlying assumptions on the product sales data are not fulfilled.

P3-112 Identifying and Modeling Meteorological Risk Factors Associated with Pre-harvest Contamination of Generic *Escherichia coli* in an Integrated Dairy and Crop Farm

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❖ ***Developing Scientist Competitor***

Introduction: Enteric foodborne pathogens can be shed, survive and multiply in the environment, thus serving as reservoirs or sources of contamination for produce during cultivation. It is necessary to investigate risk factors for pre-harvest contamination in produce farms.

Purpose: This study sought to identify specific meteorological factors affecting the presence and population levels of generic *Escherichia coli* (as an indicator for fecal contamination) in an integrated dairy and crop farm.

Methods: Over 14 months, environmental samples were collected from locations within an integrated dairy and vegetable crop farm, and enumerated for generic *E. coli*. Local weather factors were evaluated for their association with the presence of generic *E.coli* by using logistic regression and classification trees. In addition, negative binomial regression and regression tree method were applied to identify factors affecting population levels of generic *E. coli* from a sample location.

Results: The logistic regression and classification tree identified monthly precipitation (OR=4.4, $P < 0.0001$) and monthly temperature (OR=1.1, $P < 0.0001$) as risk factors, indicating that the probability of isolation of generic *E. coli* increases with an increasing average amount of rain (>1.42 mm) and increasing average temperature (>20.2°C) in the previous 30 days. However, probability of isolation was negatively correlated with rainfall amount within 2 days of sampling ($P < 0.0001$). In addition, according to the negative binomial model and regression tree, generic *E. coli* populations decreased with increasing rainfall and wind speed in the previous 2 days, suggesting that recent rainfall (>0.51 mm) and high wind speed (>2.53 m/s) may lower generic *E. coli* population levels within farm environments.

Significance: Results suggest that presence and population levels of *E. coli* on integrated dairy/vegetable farms is influenced by temperature, precipitation and wind speed. Meteorological factors should be considered when evaluating farm management practices to reduce pre-harvest pathogen contamination.

P3-113 Data Development for a Predictive Risk Assessment Model Used to Evaluate Intervention Strategies that Reduce the Burden of Foodborne Disease Caused by Human Norovirus

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Introduction: Long-term care facilities (LTCFs) are common settings for human norovirus outbreaks, and facility food services are significant contributors to disease. Development of an effective food safety plans that address key risk factors is essential.

Purpose: To develop the probabilistic and numerical data for use in a quantitative predictive risk assessment model (NorOPTIMAL) designed to simulate the spread of human norovirus contamination and disease in LCTFs, with a focus on food and surfaces.

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Methods: Key model inputs were defined for the NorOPTIMAL model, and those terms were searched in relevant electronic databases (e.g., PubMed, IngentaConnect, norocorelit.com) to identify peer-reviewed literature. Final input values were derived using data from the literature review, research conducted by the NoroCORE food safety initiative, and expert judgment.

Results: Input values and distributional data were developed for five categories of information in NorOPTIMAL, including 1) Agents (e.g., residents, health care workers, kitchen staff) and their behaviors (e.g., hand washing, food washing compliance); 2) Hazard (e.g., dose-response, food washing removal); 3) Symptoms (e.g., number of vomiting episodes, shedding rates); 4) Timeline of Activities (e.g., food preparation, cleaning events), and 5) Cost of Interventions (e.g., cost of equipment, training). For example, sanitation/disinfection compliance of food utensils varies between 0 to 91%, average 61%. Transfer of norovirus between an agent and food serving is 0 to 12% for dry conditions, and 20 to 70% for wet conditions. Finally, washing food reduces norovirus by 0.39 to 1.26 log, average 1.01 log.

Significance: This work provides a unique compilation of data describing agents and the microenvironment that determine the contamination, spread, and disease burden for human norovirus in the LTCF setting, including food preparation. These data will be used to populate the model so as to evaluate the efficacy of candidate intervention strategies and inform development of effective food safety plans.

P3-114 Applying Predictive Microbiology and Microbial Risk Assessment to Assess the Risk of Ready-to-Eat Food Products in Taiwan Based on Consumption Habits

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Introduction: In Taiwan, the major etiologic agents of foodborne illness are bacteria. Ready-to-eat (RTE) products are consumed directly without reheating and therefore, pose a higher risk to consumers than other types of foods. Mayonnaised rice is a RTE product that may be a major cause of staphylococcal poisoning in Taiwan.

Purpose: The purpose of this study was to evaluate the relative risk of staphylococcal poisoning caused by mayonnaised rice through quantitative microbial risk assessment (QMRA).

Methods: Samples of mayonnaised rice were collected from local convenience stores to test for the presence and concentration of *Staphylococcus aureus* to establish the baseline for QMRA. The storage conditions and consumption patterns of mayonnaised rice were identified. Inoculated studies were conducted to establish the growth kinetics of *Staphylococcus aureus.* The growth models were developed using the USDA Integrated Pathogen Modeling Program (IPMP 2013). Finally, QMRA was conducted using @Risk to evaluate the relative risks of staphylococcal poisoning.

Results: Dried meat floss onigiri containing mayonnaise may pose a high risk to consumers due to its frequent association with *S. aureus*. Analytical results showed that the annual average risk probability of staphylococcal poisoning caused by this product in Taiwan is 7.5×10^{-11}, and the storage temperature and the initial contamination levels are the most important two factors contributing to the risk. The safe storage time for spring, summer, fall and winter seasons are 18, 12, 16, and 34 hours, respectively.

Significance: The safest way for consumers to store mayonnaised rice is refrigeration. The retailer must properly control temperature during storage and distribution.

P3-115 A Systematic Meta-Analysis of *Toxoplasma gondii* Prevalence in Meat Animals in the United States

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Introduction: *Toxoplasma gondii* is a widely distributed protozoan parasite. It is one of the most important foodborne pathogens worldwide. The Centers for Disease Control and Prevention reported that *T. gondii* is one of three pathogens (along with *Salmonella* and *Listeria*), which together account for >70% of all deaths due to foodborne illnesses in the United States. Meat animals are reservoirs for *T. gondii* and act as one of the sources for parasite transmission to humans. Based on limited population-based data, the Food and Agriculture Organization/World Health Organization estimated that approximately 22% of human *T. gondii* infections are meatborne.

Purpose: The objective of this study was to conduct a systematic meta-analysis to provide a precise estimation of *T. gondii* infection prevalence in meat animals produced in the United States.

Methods: A comprehensive literature search was conducted to collect eligible studies for *T. gondii* prevalence in meat animals from four databases (PubMed, Google Scholar, MEDLINE and Web of Science). Prevalence was estimated in six animal categories (confinement raised market pigs, confinement raised sows, non-confinement raised pigs, lamb, goats, and non-confinement raised chickens) by a quality-effects model.

Results: A wide variation in prevalence was observed in each animal category. Animals raised outdoors or that have outdoor access had a higher prevalence as compared with animals raised indoors. *T. gondii* prevalence in non-confinement raised pigs ranked the highest (31.0%) followed by goats (30.7%), non-confinement raised chickens (24.1%), lambs (22.0%), confinement raised sows (16.7%), and confinement raised market pigs (5.6%).

Significance: The results obtained could not only allow researchers to understand *T. gondii* prevalence in different animal species, but can also be used as an important input in quantitative microbial risk assessment models to further predict public health burden.

P3-116 Validation of Predictive Risk Tools Applied to Strategic Facility Investments

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Introduction: Strategic investment decisions regarding plant improvements across the enterprise are challenging. At any given time, many potential improvements or updates could be undertaken at any one of a company's multiple facilities; however, financial resources to support these investments are limited. Extensive data is likely available through various systems, including temperature logs and microbial sampling, but how to use that data to inform decisions to best reduce risk to the enterprise is far from clear.

Purpose: The purpose of this study was to validate predictive risk tools for the purpose of evaluating strategic investments across a poultry enterprise by comparing predicted microbial levels of packaged product against laboratory measurements.

Methods: Historical plant improvements and their impact on processing conditions were quantified for three facilities over a five year window. Ambient temperature data, process logs (e.g., chlorine levels and core temperatures measured at chilling), and the prevalence and severity of incoming contamination were also mined to inform the predictive modeling. A predictive cloud-based software tool was utilized to perform ensembles of simulations for each quarter of the time window using these data as inputs in order to produce predicted contamination levels as a function of time for each facility.

Results: Comparison of model predicted microbial levels and laboratory measured microbial levels of packaged products show reasonable agreement for air packed poultry (favorable comparisons and consistently similar trends). These results indicate that potential strategic investments can be evaluated in silico based on currently available data in a manner that informed decisions that reduce risk to the enterprise.

Significance: This presentation provides a quantitative comparison of predictive model results with actual laboratory measurements that illustrate how quantitative software-based modeling tools can be applied to inform strategic facility investments based on readily available and reasonably predictable data.

P3-117 Shiga Toxin-producing *E. coli* O157:H7 Dose-Response Estimation from Outbreak Data

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Introduction: The Shiga toxin-producing *E. coli* (STEC) O157:H7 strain is frequently associated with foodborne outbreaks. Young children, the elderly, and immunocompromised individuals are most susceptible to develop illnesses, yet it is not uncommon for persons of any age to become infected.

Purpose: In order to support quantitative risk assessment of this pathogen, meta-analytical dose-response models were developed—summarizing the relationship between numbers of ingested STEC O157:H7 cells and probability of someone falling ill as a result.

Methods: After an exhaustive literature search, 14 STEC observations from 10 outbreak studies were brought together, and four classical dose-response models were adjusted: Exponential, Beta-Poisson, Weibull-gamma, and Gompertz. Variables such as population type (normal and susceptible), food matrix (categorized as water, raw food, processed meat, and cheese), mean dose, exposed population, and number of ill cases were extracted from outbreak studies. A logistic regression model with random effects placed on food matrix and weighed according to exposed persons was adjusted to assess the effect of population susceptibility, food matrix, and dose.

Results: Exponential and Beta-Poisson dose-response models had comparable measures of Bayesian Information Criterion (BIC= -21.0 and -18.7, respectively) and fitted the outbreak data better than the Weibull-gamma and Gompertz dose-response models (BIC= -16.8 and -16.2, respectively). Gompertz model was the least adequate as it overestimated probabilities of illness at low doses. The weighted logistic regression model demonstrated that both population susceptibility (P<0.0001) and food matrix (P<0.0001) had significant impact on probability of illness. For the same doses, higher probabilities of illness were obtained for the susceptible population and water. The meta-analysis logistic model was capable of depicting a relationship between dose and probability of illness, specifically for processed meat in both normal and susceptible populations.

Significance: Understanding the dose-response relationship for this pathogen will promote increased food safety and as a result, can reduce the number of foodborne illnesses.

P3-118 A Quantitative Risk Assessment for Shiga Toxin-producing *E. coli* in Raw and Pasteurized Bulk Milk Sold Directly from Producer to Consumer in the Informal Market in South Africa

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Introduction: Quantitative microbiological risk assessment and predictive modelling is an important scientific tool in the systems of food safety which provides evidence-based and transparent estimation of the risk of foodborne illnesses. The tool predicts the effects of intervention measures in food production processes and increase food safety.

Purpose: The purpose of this study was to investigate the public health risks associated with the consumption of producer-distributor (PD) bovine bulk milk and estimate the resulting burden of illness that may occur under the current production and marketing conditions in South Africa.

Methods: A total of 258 PD bulk milk samples were collected from purchase points in 8 different geographical regions around South Africa. Shiga toxin-producing *E. coli* (STEC) were isolated and identified using molecular techniques. Data for the risk models was obtained from the recently completed studies in South Africa. Inputs for the models was complemented with data from published and unpublished literature. Hazard characterization was based on dose-response from literature. A probabilistic exposure model with Monte Carlo simulation was developed taking into account prior collected prevalence data of STEC in raw and pasteurized PD bulk milk, and survey information from farms, retail and households. Sensitivity analysis for the assessment of the uncertainty and variability associated with the model was also carried out

Results: Prevalence of STEC in milk samples was 10.9 % (n=258). The study revealed that even though STEC is eliminated by pasteurization, it appears that the risk of infection persists due to post-contamination at retail stage. The baseline study quantifies the risk of STEC from informally marketed producer-distributor milk and estimates the incidence rates in SA.

Significance: Information obtained can be used to formulate risk-based mitigation strategies and policies in informal markets.

P3-119 Leveraging Seasonal Variation and Identifying Best Management Practices for Produce Brush Washer

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✦ ***Undergraduate Student Award Competitor***

Introduction: Postharvest washing equipment can serve as a vector for cross-contamination of produce after harvest. Often, older on-farm produce processing equipment is not designed with easy cleaning and sanitizing in mind. The risks of this type of equipment must first be evaluated before selecting best management practices for this existing equipment.

Purpose: This study aims to understand the risks posed by an OESCO Brush Washer, then to identify mitigating strategies to combat these risks and offer operators best management practices moving forward.

Methods: Environmental samples measuring coliforms were taken during the 2014 and 2015 growing seasons on four parts of the brush washer- the input and output chutes, the brushes and the drain area. The data were analyzed for seasonal trends and for areas with the highest potential for cross contamination to produce. These trends were then used to pose target cleaning strategies.

Results: Two season average of coliform count on the brush rollers was 5 log CFU/ml. Exact values were observed to vary based on last operation and operator. Over the summer 2015 season, coliform counts ranged from 0 to 4.5 log CFU/ml, evidence of operator inconsistencies when resetting machine for next use. The range on the brushes was the widest of all of the areas tested; hence it will be specifically targeted in the sanitation plan. Purging the machine for 5 minutes after use was shown to yield an average reduction of 1 Log CFU/ml on the brushes, indicating that a targeted cleaning plan can reduce the dramatic range of remaining bacteria.

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Significance: Identifying best management practices for postharvest farm equipment can help with extension programming to reduce risks on-farm. These data suggest that farm owners and operators using this brush washer may want to modify their best management practices, SOPs, end-of-season decommissioning plans and employee training to account for this large variability.

P3-120 Antimicrobial Ice-based Novel Meat Grinder Sanitation Process

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Introduction: An estimated 60% of all retail beef sales are in the form of ground beef. There are existing protocols and control points in meat processing, which specify the frequency and proper procedures for grinder sanitization. Current industry protocol is to disassemble and then clean meat grinders at the end each shift. However, if contamination occurs between two cleaning operations, large amounts of the product could be contaminated and subject to recall.

Purpose: This study was designed to develop a rapid intervention step to sanitize meat grinders without disassembling them.

Methods: Meat grinders were contaminated by processing 500 g beef trims spiked with either 3 or 6 log CFU/g *E. coli* O157: H7. After contamination, 600 g peroxyacetic acid ice (300 mg/L) along with 500 ml peroxyacetic acid solution (300 mg/L) were processed through meat grinders to inactivate pathogen. After antimicrobial ice treatment, two 250 g uninoculated beef pieces were ground and collected to determine transfer of pathogens from meat grinder to ground beef samples. Six hundred g deionized water ice with 500 ml deionized water treatment (DI ice), and no treatment controls were used in this study. Each experiment was repeated at least five times.

Results: When grinders were inoculated with low levels of pathogens, antimicrobial ice treatment completely eliminated *E. coli* O157: H7 cross-contamination. While DI ice and no treatment control yielded a significantly higher ($P < 0.05$) pathogen recoveries; 1.98± 0.31 and 2.82± 0.26, respectively. Antimicrobial ice treatment of meat grinders inoculated with higher levels of pathogens yielded recoveries of 1.60±0.42 log CFU/g *E. coli* O157: H7 in the ground beef. Which is significantly lower than DI ice (3.48±0.18 log CFU/g) and no treatment control (4.79± 0.41 log CFU/g)) recoveries ($P < 0.05$).

Significance: This novel method could provide a rapid alternative to the traditional meat grinder sanitation process.

P3-121 Determination of Biofilm Dispersion Using Ethylenediaminetetraacetate on Food Processing Surfaces

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Introduction: Developed a reproducible biofilm reactor to generate mixed strain replicate biofilms on High Density Polyethylene (HDPE) coupons. These replicate biofilms were then challenged against concentrations of alkaline Ethylenediaminetetraacetate with dispersants, solvents, and amphoteric surfactants. This was to determine an aid in biofilm dispersion on surfaces in conjunction with cleaning and sanitizing food contact surfaces.

Purpose: This study was to evaluate the dispersion capability of Ethylenediaminetetraacetate with adjuncts against mixed *P. fluorescens*, *L. monocytogenes*, and *Salmonella Typhimurium* based biofilms on simulated industrial food processing plant equipment.

Methods: Bacterial cultures were inoculated in diluted Tryptic Soy Broth (TSB) active flow system for 96 ± 5 h. Once the mature biofilms (> 6.0 log CFU/cm^2) were developed the HDPE coupons were removed and rinsed to eliminate any planktonic cells on the surface. This was then followed by exposure to different concentrations and mixtures of the blended compounds. Post dispersant exposure all coupons were added to D/E broth in centrifuge tubes and vortexed with sterile glass beads to suspend the remaining sessile viable cells into solution. From there the solutions were serially diluted, and plated onto Tryptic Soy Agar. Bacterial counts were compared against control coupons to determine log removal.

Results: The experimental combinations show a threshold of 1.622 g/L Ethylenediaminetetraacetate with 0.353 g/L carboxylic polymer in an alkaline solution (> 10 pH) to yield 1.0 log CFU/ cm^2 reduction. From the data we were able to determine an optimal concentration of Ethylenediaminetetraacetate for a minimum of a 3 log CFU/cm^2 removal and a maximum of a 4 log CFU/cm^2 removal at 3.245 g/L with two organic food derived amphoteric surfactants at 0.2457 g/L, 0.312 g/L and a carboxylic polymer at 0.706 g/L.

Significance: The ability to test dispersion technology on food plant biofilms is essential for developing solutions to the problem. In this experiment we were able to test replicate biofilm samples and determine an optimum formulation, which in turn can aid food processors in penetrating and removing persistent pathogenic and spoilage organism based biofilms embedded within their production surfaces.

P3-122 Nano-engineered Sanitation Surfaces for Prevention of Bacterial Adhesion

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Introduction: The bacterial adhesion mechanism is considerably complex and many factors can affect adhesion scenarios. Although many techniques have been tried to minimize the surface contamination resulting from bacterial adhesion, the effect of nanoscale surface patterns with modulated surface wettability to the bacterial adhesion was still under investigation. Cell adhesion *in vivo* is a three-dimensional (3D) phenomenon that is distinct from the interaction on two-dimensional (2D) surfaces *in vitro*. The attachment of bacterial cells is associated with various cell-surface interactions including hydrodynamic force, porosity, surface energy, and roughness. Recent advancements in fabrication have made it possible to create well-organized nanofeatures (i.e. nanoporous and nanopillared) uniformly over a large surface area of a metal specimen. However, it has not yet been studied systematically how such well-regulated nanofeatures affect the adhesion of bacteria and the formation of biofilms in various surface wetting conditions.

Purpose: This study was aimed to explore how nanoscale surface patterns with modulated surface wettability would affect the bacteria adhesion and potentially prevent biofilm formation.

Methods: Nanosmooth (control) and nanoporous stainless steel surfaces were fabricated by anodizing the degreased specimen in a 5% vol. of perchloric acid in anhydrous ethylene glycol. At this step, the back side (unpolished surface) of the specimen was covered with a water resistant tape to prevent the effect of surface irregularities on the intensity and direction of the electric field. Thereafter, the taped specimen was served as an anode and a platinum foil was used as a cathode. The applied voltage and anodization time were varied to obtain different pore diameters. In order to achieve 50 and 80 nm in pore diameters, the anodizing voltage and time combinations were 40V for 10 min and 50V for 35 min, respectively. A field emission scanning electron microscope and atomic force microscope (AFMs) with silicon tip coated with reflective aluminum coating were used to visualize the micro/nanoscale surface morphologies and determine surface roughness of the developed surfaces. For bacterial quantification, sample plates were dried in the air then a droplet of fluorescence stain namely 4,6-diamidino-2-phenylindole (DAPI) and a glass cover slip were placed on the surfaces in order to make bacterial cells easily observed under the fluorescence microscope.

Results: The presences of 50 and 80 nm nanoporous patterns significantly inhibited the adhesion of *L. monocytogenes*by 2.0 to 2.3 log-cycles, depending on the pore diameter. However, an increase in the pore diameter from 50 to 80 nm did not significantly increase the anti-adhesion effect of the nanoporous surface; hence, the 50 nm pore size which required less power consumption and time to fabricate (40V 10 min) was preferable. Both of the nanoporous surfaces were fabricated to have three-dimensional porous structures which had 'peak to valley' distances of 27 to 33 nm, depending on the pore sizes. Therefore, during adhesion test the bacterial cells were limited to contact and interact with only the peak areas of the nanofeatures. As a result, the attractive interactions between the cells and substratum, which were maximum at the cell-substratum distance of less than 15 nm, were limited and most of the cells which were not stably anchor to the surface could possibly remain in a planktonic state in the aqueous suspension.

Significance: The anodization technique developed in this study is simple, scalable, and adaptable to various types of materials so that the 3D nanopillared substrates would be of great significance in many anti-microbial applications, such as water supplies, biomaterials, and food processing, where the biofilm formation could be widespread public health problems.

P3-123 Antimicrobial Effect of Reactive Oxygen Species (ROS) Generated from Ultraviolet (UV-A) Light Exposure of Benzoic Acid

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Introduction: Benzoic acid (BA) is a commonly used preservative whose activity is based on the intracellular acidification. However, in a unique observation, we found that the exposure of 5 mM BA to UV-A (365 nm) light for 30 minutes caused more than 5±0.77-log reduction in *Escherichia coli* O157:H7 compared to 0.96±0.26-log reduction in presence of 5 mM BA in dark or 0.39±0.09-log reduction in presence of UV-A alone. We postulate that this synergistic antimicrobial activity of BA and UV-A light was due to photosensitization of BA by UV light that resulted in generation of diverse reactive oxygen species.

Purpose: To test this hypothesis, we investigated the generation of common ROS such as singlet oxygen and hydrogen peroxide, upon exposure of BA to UV-A (365 nm), B (312 nm) or C (254 nm).

Methods: Ten mM BA was exposed to UV-A (2,015 μW/cm^2 intensity), B (1,427.5 μW/cm^2 intensity) or C (4,762 μW/cm^{2}intensity) light with a same dose level. Singlet oxygen was detected by measuring Furfuryl alcohol (FFA) degradation rate by singlet oxygen using high pressure liquid chromatography (HPLC). Hydrogen peroxide generation was measured using ferrous oxidation-xylenol orange (FOX) assay.

Results: Altogether, 3.57±0.50 pM, 31.74±0.94 pM and 58.66±2.70 pM singlet oxygen and 0.06±0.01 uM, 1.69±0.10 uM and 1.35±0.06 μM hydrogen peroxide were generated from exposure of BA to UV-A, B, and C light, respectively. Thus UV exposure of BA produced diverse ROS and UVC and UVB were more efficient wavelengths than UVA in terms of ROS generation.

Significance: We envision that this synergistic interaction of BA and UV light can be harnessed for diverse applications such as advanced oxidative processes for degradation of xenobiotic compounds and inactivation of pathogenic microorganisms in foods and on food-contact surfaces.

P3-124 Thermal Sanitization Treatments for Eliminating *Listeria monocytogenes* from Industrial Mushroom Disk Slicers

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Introduction: Although there are no reported outbreaks of listeriosis associated with consumption of commercially grown fresh mushrooms, contamination can occur as evidenced by product and processing environment surveys and recalls. *Listeria monocytogenes* can colonize processing environments and transfer to food contact equipment. Industrial mushroom slicers consist of successive disks aligned and separated by spacers. This design makes complete disassembly for cleaning and sanitizing time consuming and therefore impractical.

Purpose: Although current industry practice is to remove the slicer head for submersion in hot water, this practice must be validated for elimination of *Listeria*. In this study, effective thermal sanitization treatment parameters for mushroom disk slicers were determined.

Methods: A *L. monocytogenes* cocktail, which included strains isolated from mushrooms and environmental samples, was inoculated into trypticase soy broth with yeast extract (TSBYE) or attached onto stainless steel coupons for up to 7 days. Thermal death time curves were generated by immersing samples in a circulating water bath at 50, 60, and 70°C and surviving cells were enumerated by plating onto trypticase soy agar with yeast extract (TSAYE).

Results: Planktonic cells treated immediately after inoculation were the most heat tolerant ($P \leq 0.05$) and were used to calculate *D*-values of 11.53 ± 0.36, 1.90 ± 0.04, and 0.99 ± 0.02 minutes, respectively. Complete inactivation times, evidenced by absence after enrichment, were 120, 20, 10 minutes, respectively. The theoretical cold spot within the slicer assembly, determined using numerical software (Comsol), was the inside surface of the spacers. This was confirmed by attaching thermocouples at locations within the slicer head, immersing it in a clean-out of-place (COP) tank filled with water at temperatures between 55 and 75°C, and monitoring temperatures using a data logger.

Significance: These results were used to validate thermal sanitization treatments for eliminating *Listeria monocytogenes* from mushroom disk slicers.

P3-125 Contact Time and Its Effect on Cross-contamination of *Enterobacter aerogenes* from Surfaces to Foods

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❖ ***Developing Scientist Competitor***

Introduction: The "five second rule" is based on the belief that bacteria are unable to contaminate food within five seconds after being dropped on the floor. The rule has been explored, but only to a limited degree in the published literature.

Purpose: This study quantifies cross-contamination rates of *Enterobacter aerogenes* from four common household surfaces to four representative foods considering contact time and the matrix containing the organism.

Methods: Two inoculum matrices were used: tryptic soy broth (TSB) containing an overnight culture and peptone buffer containing cells from overnight culture separated by centrifugation. Household surfaces (tile, carpet, wood, stainless steel) were inoculated with a nalidixic acid resistant strain of *E. aerogenes* and dried for 5 h. Surfaces contained ~7.0 log CFU/surface after drying. Squares (4x4 inches) of bread, bread with butter, watermelon

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and gummy squares were dropped on the respective surfaces from 5 inches and for four different times (0 to 300 sec), and the transferred cells enumerated. Each unique condition was replicated 20 times for 2,560 total measurements.

Results: The interactions of variables, as well as individual variables were analyzed for statistical significance by multiple linear regression analysis. The time, food, surface and the food*time interaction were all shown to have a significant effect ($P < 0.0001$) on the log % transfer of *E. aerogenes*. Time*surface, food*surface and matrix*surface interaction had a significant effect on log % transfer ($P \leq 0.0001$). The matrix ($P = 0.0129$) and food*matrix interaction ($P = 0.0445$) were also significant. The time*matrix interaction was not statistically significant regarding log % transfer rate ($P = 0.4949$).

Significance: All variables and their interactions play a role in the transfer rate of *E. aerogenes* from surface to food. Overall, longer contact times promote greater transfer. Although time*matrix interaction was not statistically significant, the individual variables all have a significant effect on transfer.

P3-126 Inactivation of Human Norovirus and Feline Calicivirus by Chlorine Dioxide Delivered as a Fog

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Introduction: Human norovirus is the major cause of gastroenteritis in humans worldwide, responsible for 48% of foodborne illnesses in the United States. Contact surfaces can harbor norovirus through exposure to the stool or vomit of an infected person, and transmit the virus to humans and food. Proper surface disinfection can prevent virus transmission, and reduce the burden of illness associated with outbreaks.

Purpose: To investigate the inactivation of representative human norovirus GI.6 and GII.4 Sydney strains, and the cultivable surrogate feline calicivirus (FCV), by chlorine dioxide delivered as a fog.

Methods: Virus stocks were dried on stainless steel coupons, and placed at five locations (different elevations and distances) in a 2,377 ft^3 BSL-3 containment laboratory. Using the Room Decontamination System (RDS) 3110 (AeroClave™, Winter Park, FL), chlorine dioxide (Vital Oxide, West Palm Beach, FL) was aerosolized in the room at 12.4 ppm (0.35 ml/ft^3, recommended) and 15.9 ppm (0.45 ml/ft^3). After a 10 min dwell time, viruses were recovered by elution, and log reductions in human norovirus and FCV were calculated based on genomic copies (after RNase pre-treatment) and plaque forming units, respectively. **Results** were expressed as log reduction in estimated virus concentration after treatment.

Results: For a treatment with 12.4 ppm chlorine dioxide delivered as a fog, 1.7 ± 0.2 and 0.5 ± 0.0 log reductions were observed for human norovirus GI and GII, respectively ($P<0.05$). At 15.9 ppm, log reduction was only 0.4 ± 0.1 for both GI and GII strains. Log reduction in infectious FCV was 2.4 ± 0.2 at 12.4 ppm versus 1.8 ± 0.1 at 15.9 ppm ($P=0.007$). Activity against all three viruses was uniform across various room locations.

Significance: Chlorine dioxide delivered as a fog demonstrated marginal activity against human norovirus and FCV under the parameters used in this study.

P3-127 Study of Hand-washing Methods in Malawi Utilizing Available Water and Resources to Evaluate Aerobic Plate Count, Coliforms and Generic *Escherichia coli* on Human Hands

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Introduction: Hand-washing is recognized as a defense against foodborne disease transmission. In developing countries, diarrhea is a leading cause of death in young children. Therefore, many groups have acknowledged the need for education about hand-washing in these rural developing areas.

Purpose: The objective of this study was to evaluate the effectiveness of typical hand-washing techniques in villages in Malawi.

Methods: Children and adults (n = 113) from of Malawi were assigned one of four treatment groups (water only, and water with red bar soap (RS), green bar soap (GS), or liquid soap (LS)). Objective cleanliness scores were recorded for each set of hands. A sterile swab was used to sample one hand, assigned washing treatment performed, and another swab was used on the other hand of the same individual. Water samples were collected using sterile plastic tubes (n = 3) and utensils used to transport water were swabbed prior to filling with water (n =2). All samples were analyzed for aerobic plate count (APC), coliform and generic *Escherichia coli* in duplicate. Samples were quantified at two university laboratories in Malawi.

Results: Before washing, hands averaged 5.24 log CFU/hand, with no reduction of APC from any treatment. Liquid soap was most effective at reducing coliforms from 5.29 to 4.27 CFU/hand, and generic *E. coli* from 5.42 to 2.87 CFU/hand after washing. Water samples tested averaged an APC total of 3.09 log CFU/ml and 36.33 CFU/ml of coliforms, while utensils used to transport water had an average of 7.48 CFU/swab APC indicating that they could be a source of hand contamination.

Significance: The importance of hygiene beyond a request to utilize hand-washing was made evident in this study. Further investigation and outreach is needed to educate these communities on the importance of cleaning available water and washing utensils for hand-washing to be an effective hygiene improvement.

P3-128 Efficacy of Antimicrobial Compounds in Soaps to Reduce *E. coli* and *E. faecalis* in a Soiled Hand-washing Model

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Introduction: It has been demonstrated that contaminated hands are among the main sources for transmission of foodborne pathogens. In the agricultural environment, hands could become easily contaminated during work activities caused in many cases, by poor hygiene practices.

Purpose: To evaluate the reduction of *Escherichia coli* and *Enterococcus faecalis* in soiled hands after washing with soaps with various antimicrobial compounds, and determine microbial survival in rinsates.

Methods: Four commercial soaps with different antimicrobial compounds were analyzed. *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 19433 were inoculated (10^3 and 10^6 CFU/g, final concentration) on sterilized agricultural soil. Previously-decontaminated hands were put in contact with soil for 2 min, and immediately washed with liquid soaps with and without antimicrobial compounds (non-antimicrobial bland soap, triclosan, citric extracts, chloroxylenol, chlorhexidine gluconate and distilled water (control). Remained bacteria on hands were determined by a membrane filtration method using selective media (Rapid *E. coli* 2 Agar and *Streptococcus* KF Agar plates). Hand rinsates were collected and incubated for 20 h at 25°C, and viable bacteria were determined at various times. **Results** were analyzed with NCSS, LLC Version 6.0.

Results: Washing hands with soap with chlorhexidine gluconate provided the lowest concentration of *E. coli* remained per hand ($P<0.05$) (3.17 ± 0.0 log CFU [99.9% of reduction] and 4.17 ± 0.2 log CFU [99.99% of reduction]), when inocula of 10^3 and 10^6 CFU/ml were applied, respectively. Similarly, for *E. faecalis* the higher reduction was obtained with chlorhexidine gluconate, [3.0 log CFU to 3.15 ± 0.3 log CFU (99.9% of reduction)] followed by

chloroxylenol [6.0 log CFU to 4.43 ± 0.3 log CFU (99.99% of reduction)]. In most cases, the level of viable bacteria in rinsates remained constant during the period analyzed.

Significance: This study shows the efficacy of antimicrobial soaps in soiled hands which can be used to reduce contamination during handling products.

P3-129 Development of Decision–support Systems Based on Physico-chemical and Microbiological Data for Improvement of the Quality and Safety of *Aloreña de Málaga* Table Olives

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Introduction: Table olives are one of the most representatives and consumed fermented vegetables in Mediterranean countries. In Spain, *Aloreña de Málaga* table olive has a Protected Designation of Origin. However, there is an evident lack of standardization of elaboration processes and HACCP systems thus implying the need of establishing decision-support tools that allow their commercialization and shelf-life extension.

Purpose: The present work aims at developing a decision-making tool to standardize the HACCP system of *Aloreña de Málaga* table olives based on the identification of potential hazards or deficiencies in hygienic processes for the subsequent implementation of corrective measures.

Methods: Three representative companies were visited to collect samples from food-contact surfaces, olive fruits, brines, environmental control, olive dressings, water tank, and final product. A quantification system based on a percentage of accomplishment (P_{accp}0-100%) was developed based on a series of microbiological and physicochemical criteria as well as the relative importance of each elaboration step on total hygienic quality. ANOVA statistical analyses were conducted to evaluate significant differences ($P<0.05$) between obtained measurements.

Results: The calculated P_{accp} highlighted an increasing trend in the scored values as long as the elaboration process flows towards the final product (i.e., cracking step (P_{accp}=20%); packaging step (P_{accp}=70%). Presence of *Staphylococcus aureus* was obtained in intermediate fruits and olive dressings, which were identified as potential contamination sources if they are added as raw ingredients. Washing and cracking steps were identified as critical since high aerobic and *Enterobacteriaceae* counts were obtained (3-4 log log CFU/g). Significant differences were denoted between food companies ($P < 0.05$) regarding their implementation of HACCP systems.

Significance: The present work brings further the development of an easy-to-use, flexible and useful tool for the *Aloreña de Málaga* table olive food sector and can be potentially applied to other industries and vegetable products.

P3-130 Effect of Turbidity on Chlorine Disinfection of *E. coli* O157:H7 and *Salmonella* in Leafy Green Wash Water

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Introduction: Prevention of microbial cross-contamination in produce wash water is critical in the prevention of foodborne disease outbreaks. The most commonly used sanitizer for produce washing is free chlorine, which is known to be affected by the pH and temperature of the wash water. However, the organic load in wash water increases with the cumulative amount of produce processed through a flume, and this increased organic loading may affect the efficacy of free chlorine in wash water.

Purpose: The purpose of this study was to evaluate the effect of turbidity (as an indicator of organic load) on free chlorine inactivation of *Salmonella* and *E. coli*O157:H7 strains in leafy green wash water.

Methods: Chlorine disinfection experiments were carried out for *Salmonella* and *E. coli* O157:H7 using leafy green wash water at varying turbidity levels. Wash water from three commercial produce production facilities was collected at maximum turbidity after washing cut iceberg lettuce and diluted to create a range of turbidity levels for testing. Experiments were conducted at 5°C and pH 6.5-7, depending on the water source. Free chlorine concentrations were monitored throughout the course of each experiment to account for depletion. *Salmonella* and *E. coli* O157:H7 concentrations were enumerated by the most probable number (MPN) technique. Inactivation data for each microbe and water source was compared at the varying turbidity levels to determine the effect of increasing turbidity on chlorine disinfection efficacy.

Results: The inactivation data generated from disinfection experiments showed that as turbidity increased in the wash water, chorine disinfection efficacy decreased. Median inactivation rates for maximum turbidity water (40-80 NTU) were 3.5 and 6 times slower for *Salmonella* and *E. coli* O157:H7, respectively, compared to non-turbid wash water.

Significance: The results of this study can help guide produce production facilities to develop wash water management approaches and water quality monitoring strategies based on turbidity.

P3-131 Engineering of Chitosan-driven Nanoparticles to Enhance Antimicrobial Activity against Foodborne Pathogen *Escherichia coli* O157:H7

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❖ ***Developing Scientist Competitor***

Introduction: Chitosan nanoparticles (CN) have been developed as a natural antimicrobial agent with broad-spectrum antimicrobial activity. Many different types of CN have been generated using a variety of chitosan sources, cross-linkers, and sonication conditions. However, not many previous studies have comparatively assessed the antimicrobial activity of different types of chitosan nanoparticles against foodborne pathogens.

Purpose: The purpose of this study was to evaluate different engineering methods for production of CN to enhance its antimicrobial activity, which will help optimize the potential for further application of these particles.

Methods: CN was prepared in solution using different molecular weights of chitosan, cross linkers (sodium sulfate or tripolyphosphate) and sonication conditions. The size of CN was measured using a nanoparticle analyzer. The antimicrobial activity of CN was assessed against *E. coli* O157:H7 to determine the optimal conditions for chitosan nanoparticle generation with high antimicrobial properties.

Results: It was observed that CN with a size smaller than 150 nm exerted better antimicrobial activity. In addition, the selection of sodium sulfate as a crosslinker over sodium tripolyphosphate achieved better results, as most bacteria were killed after 4 h and no regrowth was observed after 24 h. For sonication power, 60 W enhanced the antimicrobial activity compared with 96 W. The best engineering conditions that enhanced antimicrobial activity included a combination of low molecular weight chitosan with sodium sulfate as cross-linking agent at a final concentration of 0.4-0.6 %.

Significance: The optimized engineering of CN particles may be applied in future studies to assess their applicability in different fields, such as animal disease treatment as well as elimination of other pathogens from food and the environment.

P3-132 Characterization and Antimicrobial Resistance of *Listeria monocytogenes* Isolated from Food and Food-related Environments

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Introduction: *Listeria monocytogenes* causes human listeriosis and is widely-distributed in the environment and in foods. The widespread use of antimicrobials can result in resistant bacterial populations. In food animals, antimicrobials are used to control and treat bacterial infections as well as for growth enhancement. An undesired consequence of this is the development of antimicrobial-resistant zoonotic foodborne bacterial pathogens and their subsequent transmission to humans through contaminated foods.

Purpose: The objective of this study was to determine the prevalence of antimicrobial resistance in *Listeria monocytogenes* strains isolated from food and food-related environments in the US.

Methods: Forty-two unrelated strains of *L. monocytogenes* were recovered from approximately 1300 food and food processing environmental samples between 2007 and 2011 as part of the FDAs pathogen surveillance program. Environmental isolates (*n*=19) and food isolates (*n*= 23) were characterized by serotyping and subtyping, antimicrobial resistance determinants, pulsed-field gel electrophoresis (PFGE), and plasmid profiles.

Results: The predominant serovars of *L. monocytogenes* from foods were 1/2a and 1/2b, but from the environment serovars 1/2a and 4b predominated. PFGE using *Asc*I digested total DNA showed genetic diversity; there were 24 PFGE pulse-types and 8 PFGE groups. All strains were susceptible to erythromycin, vancomycin, ciprofloxacin, and chloramphenicol, but resistant to nalidixic acid. Eight (42.1%), six (31.6%), and four (21.1%) environmental strains were resistant to streptomycin, tetracycline, and ampicillin, respectively. Five (26.3%) strains were resistant to 3 or more antimicrobial classes. Six out of seven tetracycline-resistant strains were recovered from food-related environments, whereas only one was recovered from food. These six tetracycline-resistant strains were serotype 1/2a, and only *tetM* was amplified from the chromosomal DNA. Two of resistant environmental isolates contained plasmids, ranging from 25 to 90 kbp.

Significance: These results suggest that epidemiological and antimicrobial resistance monitoring data from food-processing environments may be used to reduce the risk for human listeriosis resulting from the transmission of the pathogen to food products.

P3-133 Inhibition of *Listeria monocytogenes* on Deli Slicers and Food Contact Surfaces with Lactic Acid Bacteria

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Introduction: *Listeria monocytogenes* is a pathogen of concern for the food industry given its capacity to withstand standard sanitation regimes, to form biofilms, and to survive and grow in moist, refrigerated environments.

Purpose: To estimate the quantitative reduction of *L. monocytogenes* on stainless steel surfaces.

Methods: Six deli slicers and twelve unsliced deli meat were procured commercially. Six meat chubs were submerged into a three-strain cocktail of *L. monocytogenes* at 10^6 CFU/ml and were air-dried for 30 minutes. Each deli meat chub was sliced separately in a separate slicer. Three of the slicers were sprayed with 10 ml of a cocktail of lactic acid bacteria at a concentration of 10^9 CFU/ml, and were allowed to air-dry for an hour. The other six uninoculated deli meat chubs were sliced separately in each slicer. The blade and table of each slicer was swabbed with EZ Reach pre-hydrated sterile sponges. All swab samples were plated onto Modified Oxford Listeria Selective Agar. Typical colonies were counted after incubation for 18-24 hours at 35 ± 1 C. The experiment was replicated three times.

Results: The estimated reduction of *L. monocytogenes* on the slicer's table was approximately 0.9 log/100 cm^2. On the other hand, there was not any growth estimated on the blade in both the control and the treatment indicating that the inoculated meat did not transfer any pathogen to the blade as it did the table.

Significance: The use of lactic acid bacteria as a biocontrol method for *L. monocytogenes* may help reduce the burden of this pathogen in the food industry and minimize the risk of contamination of deli meat and associated food-contact surfaces.

P3-134 Comparison of Commercially Available and Novel Lactic Acid Bacteria (L28, FS56) as Bio-Sanitizers to Inhibit *Listeria monocytogenes* on Stainless Steel Surfaces

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✦ ***Undergraduate Student Award Competitor***

Introduction: *Listeria monocytogenes* is known to have the ability to attach and form biofilms on many surfaces including stainless steel. Biofilm is not easily removed with common chemical sanitizing methods used in the industry. Therefore, finding innovative ways to inhibit *Listeria monocytogenes* growth and biofilm formation is necessary.

Purpose: The purpose of this experiment was to evaluate a novel (L28) Lactic Acid Bacteria and commercially available (FS56) Lactic Acid Bacteria in inhibition of *Listeria monocytogenes* (N1-002) on stainless steel coupons at room temperature.

Methods: Sterile stainless steel coupons (2 cm x 2 cm) were placed into 6-well plates with 2 ml of *Listeria monocytogenes* (log 5.00 CFU/ml) and incubated 24 hours for attachment. After the 24 hours the *Listeria monocytogenes* was removed and each treatment and control were added. The treatments were with strains L28, FS56 at a concentration of log 8.00 CFU/ml and the control was with a blank of de Man, Rogosa and Sharpe (MRS) Broth. The *Listeria monocytogenes* counts were evaluated on modified oxford agar.

Results: Our results indicate statistical differences (P<0.05) among all of our treatments and our control for counts of *Listeria monocytogenes.* By the end of the 24 hours the MRS control had increased to log 5.76 CFU/cm^2 of *Listeria monocytogenes*. For the treatments, FS56 and L28 had log reduction of 3.1 CFU/cm^2 and 5.76 CFU/cm^2, respectively. The L28 Lactic Acid Bacteria was so effective that the *Listeria monocytogenes* was not detectable by means of direct agar plating method indicating it is more effective than the FS56 which is currently commercially available.

Significance: It is important to optimize these cultures for maximum impact and the new culture is more effective than the current commercial strains. LAB can be provided to processors in various forms (frozen, liquid or freeze-dried) and application can be easily implemented into current operations.

Poster

P3-135 Control of *Listeria monocytogenes* in Cured and Uncured Hotdogs Stored at 40°F for 150 Days Using Cultured Cane Sugar and Vinegar

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Introduction: *Listeria monocytogenes* is a gram-positive bacteria that can contaminate RTE meat and poultry products. *L. monocytogenes* is able to grow under refrigeration conditions in which RTE meat and poultry product are stored during shelf life. The shelf life requirement for both uncured and cured hotdog products are increasing and there is a need for validated natural antimicrobial which can provide protection against *L. monocytogenes* throughout the extended storage period. Verdad® N64 is a cane sugar fermentation based product which contains organic acids, small peptides and residual sugars, and vinegar and has excellent antimicrobial properties.

Purpose: To evaluate the antimicrobial performance of Cultured Cane Sugar and Vinegar product (CSV) for controlling the growth of *Listeria monocytogenes* in cured and uncured hotdogs and vacuum packaged and stored at 40°F for 150 days.

Methods: Ground beef trim, salt and spices were mixed and emulsified before stuffing in cellulose casings using different formulations and smoked. The hotdogs were divided into 25 g, surface inoculated with 5 strain *L. monocytogenes* cocktail, were individually packaged and incubated at 40°F. Samples were analyzed every 2 weeks till 150 days storage for *L. monocytogenes* counts.

Results: Inoculation level of 2.18 log CFU/g of *L. monocytogenes* was achieved on day 0 for all the treatments. Uncured and Cured Hotdogs with no antimicrobial exhibited fastest growth and reached 6.48 and 5.33 log CFU/g counts on day 14, respectively. Incorporation of 3.5% CSV in both uncured and cured hotdogs suppresses the growth of *L. monocytogenes* to the inoculation levels throughout the storage period of 150 days at 40°F.

Significance: This research demonstrates the antimicrobial performance of CSV in controlling the outgrowth of *L. monocytogenes* in both uncured and cured hotdogs for 150 days at 40°F.

P3-136 Efficacy of Buffered Vinegar to Control Outgrowth of *Listeria monocytogenes* on Natural Uncured Ham Steaks and All-pork Frankfurters during Extended Refrigerated Storage

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Introduction: The market for naturally-produced, ready-to-eat (RTE) meats has increased considerably over the last decade. However, producing this category of RTE meats without inclusion of antimicrobials may support the outgrowth of foodborne pathogens, such as *Listeria monocytogenes* (Lm), over the anticipated shelf life. Thus, additional research is needed to continue developing and validating interventions for controlling Lm on both traditional and clean label RTE meats.

Purpose: Monitor viability of Lm on uncured ham steaks and uncured pork frankfurters containing added vinegar, subjected or not to high pressure processing (HPP), during extended refrigerated storage.

Methods: One batch of freshly-manufactured, vacuum-packaged, uncured pork frankfurters and one batch of uncured ham steaks were formulated with and without dry buffered vinegar (0.74%; Verdad Powder N6) or liquid buffered vinegar (2.41%; E(lm)inate V), respectively. Products were surface inoculated with 0.5 ml of the five-strain cocktail of Lm to achieve a target level of ca. 3.5 log CFU/package. After vacuum-sealing, the packages were or were not pressurized at 600 MPa (87,000 psi) for 3 min. Ham steaks and pork frankfurters were then stored for up to 180 days at 4°C.

Results: When both products were formulated without vinegar, Lm numbers increased by ca. 6.5 log CFU/package over 180 days of storage at 4°C; however, when both products were formulated with vinegar, pathogen numbers remained relatively unchanged during extended storage at 4°C. In addition, regardless if uncured ham steaks or uncured pork frankfurters were formulated with or without vinegar, HPP delivered an initial reduction in pathogen numbers of ca. 2.2 log CFU/pack. Thereafter, pathogen numbers remained relatively unchanged after 180 days at 4°C.

Significance: Inclusion of buffered vinegar in the formulation of uncured ham steaks or uncured pork frankfurters, alone or in combination with HPP, was an effective clean label antimicrobial agent for controlling outgrowth of Lm during extended storage at 4°C.

P3-137 Internal pH and Membrane Potential of Acid Sensitive and Resistant *Escherichia coli* O157:H7 Strains under Acetic and Sorbic Acid Stress

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❖ ***Developing Scientist Competitor***

Introduction: Preservative addition to acidified foods have shown to decrease 5-log reduction times of foodborne pathogens. Acetic and sorbic acids have similar pKas. For a given external pH, each acid should accumulate to the same concentration and acidify intracellular pH. However, sorbic acid kills cells faster.

Purpose: To determine if differences in lethality between acetic and sorbic acids are influenced by changes in internal cell pH and membrane potential (delta psi), using acid sensitive and acid resistant *E. coli* O157:H7 strains.

Methods: Acid sensitive (B201) and resistant (B241) strains of *E. coli* O157:H7 were grown in glucose containing minimal medium (M9GT) and Luria broth (LBG) to induce acid resistance, than incubated with acetic or sorbic acid at pH 3.5. Internal pH and delta psi were measured with radiolabeled benzoate (internal pH) or tetraphenylphosphonium bromide (delta psi) and tritium (cell volume). Partitioning of labeled compounds between the cell pellet and supernatant were determined using a scintillation counter.

Results: The internal pH of B201 and B241 were not significant between acid treatments for cells grown in LBG, but were (P>0.05) for M9GT grown cells. Sorbic acid treatments (but not acetic acid) decreased internal pH to 6.4 and 6.6 from 6.8 and 7.1 controls, and decreased delta psi to -81.9 mV and -72.1 mV (for B201 and B241, respectively) in M9GT. A positive correlation was seen between decreasing internal pH and delta psi, r^2=0.8088.

Significance: Sorbic acid may diffuse across the cell membrane more efficiently than acetic acid which would reflect the lower internal pH reported. Additionally, the negative delta psi likely attracts extracellular protons to cross the cell membrane, killing cells faster. Although acetic and sorbic acid should affect cell death the same, these acids behave differently. Understanding the mechanisms by which organic acid preservatives kill pathogens may help define additional safety parameters for acid and acidified foods.

Poster

P3-138 Effect of Acetic Acid-based Antimicrobial Ingredients to Control Outgrowth of *Listeria monocytogenes* on Frankfurters during Extended Refrigerated Storage

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Introduction: Validation of alternative types, concentrations, and/or blends of antimicrobial ingredients as substitutes for traditional food grade antimicrobials is critical for controlling outgrowth of *Listeria monocytogenes* on ready-to-eat meat (RTE) meat and poultry products, especially in the event of post-process contamination.

Purpose: Validate the efficacy of acetic acid-based antimicrobials as ingredients to control *L. monocytogenes* inoculated onto commercially-produced frankfurters.

Methods: Pork-beef-water frankfurters were formulated by a cooperating processor with or without 0.9%, 1.15%, or 1.40% of buffered vinegar [BV; e(Lm)inate V], a blend of buffered vinegar and potassium lactate [VL; e(Lm)inate VL], or a blend of potassium lactate, potassium acetate, and sodium diacetate [LAD; e(Lm)inate LAD], or 2.37% of a blend of potassium lactate and sodium diacetate [Klac; Ultralac KL 564]. Frankfurters were surface inoculated with 2 ml of a five-strain mixture of *L. monocytogenes* to achieve a target level of ca. 4.3 log CFU/package. The packages were then vacuum-sealed and stored at 4°C for up to 120 days.

Results: In the absence of any antimicrobials, pathogen numbers increased by ca. 6.0 log CFU/package after 120 days at 4°C. When antimicrobials were included in the formulation, the higher the concentrations used, the greater the observed inhibition of *L. monocytogenes*. When frankfurters were formulated with 0.9% of BV, VL, or LAD, pathogen numbers increased by ca. 1.0 to 4.0 log CFU/package over 120 days at 4°C, whereas when formulated with 1.15% of BV, VL, or LAD pathogen numbers remained relatively unchanged. In contrast, pathogen numbers decreased by ca. 0.7 to 1.2 log CFU/package when frankfurters were formulated with 1.4% of BV, VL, or LAD or 2.37% of Klac.

Significance: Inclusion of 1.15 or 1.4% of BV, VL, or LAD as ingredients in frankfurters was equally effective as 2.37% Klac at suppressing outgrowth of *L. monocytogenes* during extended refrigerated storage.

P3-139 Application of Phage Endolysin PlyP100 in the Control of *Listeria monocytogenes* in Queso Fresco

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Introduction: Queso Fresco, a widespread Hispanic-style cheese, possesses characteristics that contribute to increase *Listeria monocytogenes*-associated food safety risk. The quest for new natural antimicrobials has shown promising potential application of endolysins (phage-encoded bacterial cell wall lytic enzymes) to food industry.

Purpose: Determine the effectiveness of *Listeria* phage endolysin PlyP100 against *L. monocytogene*s when incorporated into Queso Fresco.

Methods: His-tagged PlyP100 was overexpressed in *Escherichia coli* and subsequently purified. PlyP100 was added to miniature Queso Fresco achieving 10 U/g. Antilisterial activity of PlyP100 was tested by inoculating cheese curds with approximately 5 log CFU/g of *L. monocytogenes* cocktail, and survival of pathogen was measured during 28 days of storage at 4°C. Different inoculum sizes of *L. monocytogenes* cocktail were evaluated to determine the pathogen population killed by PlyP100 in cheese after 7 day of storage. The stability of PlyP100 in cheese was assessed by inoculating approximately 5 log CFU/g of *L. monocytogenes* cocktail on cheese surfaces at 0 – 28 days after manufacture, and pathogen survival was measured at 7 days after cheese inoculation.

Results: PlyP100 in Queso Fresco reduced viable counts of *L. monocytogenes* by approximately 0.5 log CFU/g and remained without regrowth during 28 day storage. Furthermore, PlyP100 was able to achieve no *L. monocytogenes* recovery after cheese enrichment when inoculation size was 1 log CFU/g. Finally, an approximate 0.5 log reduction of *L. monocytogenes* occurred regardless of whether the culture was added immediately upon manufacture or up to 14 days later, suggesting that PlyP100 remains active in cheese during refrigerated storage.

Significance: Our results support the efficacy of phage endolysins as a practical biocontrol measure against *Listeria* contaminants in Queso Fresco.

P3-140 Efficacy of Antimicrobials and Their Combinations in Controlling *Listeria monocytogenes* in Broth and Milk Systems

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❖ ***Developing Scientist Competitor***

Introduction: Dairy-related recalls and illnesses are often attributed to *Listeria monocytogenes* contamination of high-risk cheeses characterized by high water activity and pH levels. Although post-lethality interventions have been identified for more acidic foods, control options are limited for products where heating, controlling pH, and/or controlling water activity is not practical.

Purpose: The objective of this study was to determine the efficacy of GRAS antimicrobials, both singly and in combination, to control *L. monocytogenes* in a near-neutral broth system and in milk.

Methods: Broth micro-dilution checkerboard assays were utilized to identify minimum inhibitory (MIC) and bactericidal concentrations (MBC) of antimicrobials against *L. monocytogenes* in growth media (pH ~7.4) incubated at 37°C for 24 h. Fractional inhibitory and bactericidal concentration indices (FIC_i and FBC_i) were calculated to characterize antimicrobial interactions as additive, synergistic, or antagonistic. MICs and MBCs were also determined in UHT milk stored at 7°C for 21 days using a similar dilution assay.

Results: When applied singly in broth, lauric arginate (LAE), hydrogen peroxide (HP), and e-polylysine (EPL) were most effective with MICs of 6.25, 40, and 20 ppm, and MBCs of 25, 50, and 60 ppm, respectively. At 200 ppm, EPL inhibited the growth of *L. monocytogenes* in milk throughout storage, while HP (200 ppm) rapidly reduced pathogen levels to below the detection limit, which remained undetectable for 21 days. Combinations of sodium octanoate (SO) with either EPL (FIC_i 0.406) or acidified calcium sulfate with lactic acid (ACSL) (FIC_i 0.479) worked synergistically to inhibit *L. monocytogenes* in broth and synergistic bactericidal activity was identified for combinations of LAE/EPL (FBC_i 0.286) and SO/EPL (FBC_i0.426).

Significance: Together, these results at near neutral pH and within a milk matrix serve as a basis for the identification of antimicrobials and their combinations for use in post-lethality treatments to control *L. monocytogenes* in high-risk cheese and similar products.

Poster

P3-141 Susceptibility of *Listeria monocytogenes* ATCC 7644 to Nisin Combined with Organic Acids on Fresh-cut Tomato (*Lycopersicon esculentum*) under Different Storage Temperatures

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❖ ***Developing Scientist Competitor***

Introduction: *Listeria monocytogenes* is a ubiquitous pathogen of public health and economic concern in food safety parlance. The use of conventional decontaminators such as chlorine-based antimicrobials in fresh produce has been compromised by development of resistant mutants, and health-based etiologic effect, giving rise to the use of natural antimicrobials which are eco- friendly in nature.

Purpose: The purpose of this study is to investigate the effect of nisin and its combination with organic acids in controlling *Listeria monocytogenes* ATCC 7644 on fresh-cut tomato under different storage temperatures (4, 10 and 25°C).

Methods: Artificially inoculated tomato slices (10^8 CFU/ml) were dipped in nisin alone (5,000 IU/ml) and in combination with organic acids (acetic and citric acid at 3 and 5% concentration, respectively). Chlorine solution (sodium hypochlorite) at 200 mg/L chlorine was used as control. Stored slices were monitored for 6 days. Antimicrobial treatment effect was also investigated on physicochemical parameters (pH, total soluble solid, titratable acidity, vitamin C and Chroma values-CIE L* a* b*) at 4°C.

Results: After six days of storage along the storage temperature, nisin dip statistically ($P \leq 0.05$) resulted in 1 to 3-fold log reduction, while nisin treatment with citric acid had no significant difference. Control treatment was comparatively least effective. Treatment with acetic acid resulted in ~3 to 4-fold log reduction. Antimicrobial treatments resulted in lowered pH, reduced total soluble solids and total titratable acidity at 10 and 25°C. However, vitamin C content increased with no significant change in the color consistency of the tomato slice

Significance: The result of this study showed the promising potential synergy of nisin with organic acids in controlling *L. monocytogenes* on fresh tomato slices and also to maintain its keeping quality.

P3-142 Combinations of Multiple Natural Antimicrobials with Different Mechanisms as an Approach to Control *Listeria monocytogenes*

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❖ ***Developing Scientist Competitor***

Introduction: To achieve synergistic interaction of antimicrobials likely requires that individual compounds have different mechanisms of inhibition or inactivation. Natural antimicrobials vary in reported mechanisms of inhibition of bacterial cells. Finding a combination of natural antimicrobials that acts synergistically would allow a reduction in the use concentration which is important for essential oils (EO) or EO components as they may contribute undesirable sensory effects to foods.

Purpose: The objective of this study was to attempt to achieve synergistic antimicrobial interactions and reduce use concentrations by combining EOs and a naturally occurring hydroxycinnamic acid with reported different mechanisms.

Methods: Oregano essential oil (OEO), basil essential oil (BEO), coriander essential oil (CEO), and ferulic acid (FA) were evaluated alone and in combination against *Listeria monocytogenes* at pH 6.0 and 25°C. A broth dilution assay was used to determine the minimum inhibitory concentrations (MIC) of individual and combined antimicrobials. Fractional inhibitory concentrations (FIC) were calculated and the interactions interpreted as synergistic (FIC ≤0.5), additive (FIC ≥0.5), or antagonistic (FIC >1.5).

Results: MICs of EOs and FA alone were 250 ppm OEO, 2500 ppm CEO, 7500 ppm BEO, and 5000 ppm FA. Binary combinations of OEO+BEO, CEO+BEO, and BEO+FA resulted in a synergistic effect (FIC ≤ 0.5). An additive effect was observed with binary combinations of OEO+CEO, OEO+FA, and CEO+FA. For tertiary combinations, synergistic antimicrobial effects were noted for all combinations except OEO+CEO+FA which had an FIC of 1.0. The quaternary combination of OEO+CEO+BEO+FA was inhibitory at 31.25, 312.5, 937.5, and 625 ppm, respectively, and had a synergistic effect.

Significance: Combining natural antimicrobials with suggested different mechanisms may be a solution for controlling foodborne pathogens and reducing use concentrations. A quaternary antimicrobial blend reduced the concentration of each compound needed for inhibition by 87.5% which could also reduce the potential for negative sensory effects.

P3-143 Serotype and Antimicrobial Resistance Distribution of *Salmonella* spp. in China during 2007 to 2012

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❖ ***Developing Scientist Competitor***

Introduction: Salmonellosis is a major public health concern worldwide. The dramatic increase of multi-drug resistant (MDR) pathogens caused by misuse of antimicrobial agents has already posed a great threat to food safety and public health.

Purpose: To better understand the prevalence and antimicrobial resistance of *Salmonella* in retail foods in China, 1,503 *Salmonella* isolates collected in five years (2007, 2008, 2010, 2011, and 2012) were characterized by serotyping and antimicrobial susceptibility.

Methods: Isolates recovered from retail chicken, beef, fish, pork, dumplings, cold dishes, and salmonellosis were isolated based on the method of Microbial Food Safety Laboratory at NWAFU. Serotypes and antimicrobial susceptibility were determined by slide agglutination and the agar dilution method according to the White-Kauffmann classification scheme and CLSI standards, respectively.

Results: Among the 1,503 isolates, a total of 129 serotypes were diagnosed. *Salmonella* Enteritidis (21.5%), *Salmonella* Typhimurium (11.0%), *Salmonella* Indiana (10.8%), *Salmonella* Thompson (5.4%), and *Salmonella* Derby (5.1%) were most prevalent ones. Multi-drug resistance could be commonly detected among *Salmonella* isolates. A total of 1,344 (89.4%) isolates resisted to three or more antimicrobial agents. Resistance was most frequently detected among sulfisoxazole (78.1%), followed by tetracycline (70.6%), trimethoprim/sulfamethoxazole (68.0%), and nalidixic acid (63.4%). Lower resistance rates were found among gatifloxacin (17.9%), ceftriaxone (17.7%), and cefoxitin (13.2%). Different from *Salmonella* Enteritidis and *Salmonella* Typhimurium isolates, those of *Salmonella* Indiana, *Salmonella* Thompson, and *Salmonella* Shubra were more resistant to antimicrobial agents. Resistance to fluoroquinolones was most frequently found among *Salmonella* Shubra and *Salmonella* Indiana isolates, while to cephalosporins was predominantly detected among *Salmonella* Thompson isolates.

Significance: Our results highlighted the diversity of the serotype and phenotype of multi-drug resistant (MDR) *Salmonella* isolates, and indicated the complexity and importance for acquiring those serovars of the highest epidemiological data.

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P3-144 Antimicrobial Resistance of *Salmonella enterica* Environmental Isolates from the Eastern Shore of Virginia

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Undergraduate Student Award Competitor

Introduction: Tomatoes grown on the Eastern Shore of Virginia [ESV] have been associated with four laboratory confirmed *Salmonella* outbreaks. Pulsed field gel electrophoresis has implicated the same pulsotype pattern (JJPX01.0061) of *Salmonella enterica* serovar Newport in these ESV outbreaks. The FDA has further isolated the JJPX01.0061 in the farms, ponds, irrigation waters, and other locations across the ESV.

Purpose: The purpose of this study was to identify antibiotic resistance profiles of *S.* Newport isolates recovered from irrigation water and poultry litter amended soil on ESV.

Methods: *Salmonella* spp. were isolated from irrigation ponds and wells of four vegetable farms and 14 farms fertilized with chicken manure on ESV in 2015. Antimicrobial susceptibility of 301 isolates from irrigation ponds (171), irrigation wells or creeks (49), and soils (81), was tested using 96-well Sensititer Gram Negative Narms Plates (Thermo Fisher Scientific, Waltham, MA). Antibiotic-resistant strains were defined according to the National Committee for Clinical and Laboratory Standards criteria.

Results: Of the 301 *Salmonella* isolates, 19.0% (*n*=57) were resistant to at least one of the tested antibiotics, including 10 isolates from pond water, 4 from well/creek water, and 43 from soil samples. Tested isolates were resistant to tetracycline (*n*=39; 13%), streptomycin (*n*=10; 3%), ceftriaxone (*n*=7, 2%), ampicillin (*n*=4, 1.3%), amoxicillin/clavulanic acid 2:1 (*n*=3; 1%), and cefoxitin (*n*=1, 0.3%). Seven isolates (2%) were resistant to two antibiotics, mostly tetracycline and streptomycin. Four isolates (1.3%) were resistant to more than two antibiotics; these isolates, recovered from chicken manure amended soils, were resistant to 3-7 antibiotics.

Significance: The varying degrees of antimicrobial-resistant *Salmonella* isolates recovered from localized areas in ESV is concerning. The discovery of such *S. enterica* isolates in small communities can impact the supply chain and future farm tracebacks with regards to the new FSMA regulations.

P3-145 The Mechanisms of Fluoroquinolone Resistance in *Escherichia coli* from Swine Feces

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Developing Scientist Competitor

Introduction: Fluoroquinolones (FQ) are potent synthetic antimicrobials against most Enterobacteriaceae, including *E. coli*, and these agents have been extensively used in animal industry, as feed additives, as well as in veterinary and human medicine. Consequently, FQ resistance has rapidly increased worldwide posing a serious threat to the public health.

Purpose: The study was designed to investigate the frequency and mechanisms of FQ resistance in *E. coli* from swine industry. The 3 major FQ resistance mechanisms investigated in the current study were target mutations in quinolone resistance determining regions (QRDR), presence of plasmid-mediated quinolone resistance (PMQR) genes, and increase in efflux pump activity.

Methods: For 171 *E. coli* isolates (collected from 237 swine fecal samples), ciprofloxacin (CIP) resistances were screened by standard disk diffusion method and minimum inhibitory concentrations (MICs) were determined by broth microdilution method. PCR and sequencing analysis were used to confirm target mutations in QRDRs and the presence of PMQR genes. Organic solvent tolerance (OST) assay was used to measure efflux pump activity in the isolates.

Results: Of 171 *E. coli* isolates, 59 (59/171; 34.5%) showed resistance to CIP (MICs: 4 μg/ml - 256 μg/ml). Of 59 CIP-resistant isolates, 58 (98.3%) had single (*n*=1, 1.7%) or multiple amino acid substitutions (*n*=57, 96.6%) in QRDRs, whereas 9 isolates (15.3%) had PMQR genes; *qepA* (*n*=1, 1.7%), *qnrS*, (*n*=7, 11.9%), *aac-(6')-Ib-cr* (*n*=1, 1.7%), respectively. The OST assay is currently under progress.

Significance: FQ resistance mechanisms observed in animal isolates are identical to those found in human isolates. Therefore, use of FQ in animal industry should be carefully managed to prevent the dissemination of FQ-resistant bacteria from animals to humans.

P3-146 Seasonal Prevalence, Antimicrobial Resistance, and Molecular Characteristics of *Salmonella* spp. Isolated from Chicken Carcasses

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Developing Scientist Competitor

Introduction: Foodborne diseases caused by non-typhoid *Salmonella* represent an important public health problem worldwide.

Purpose: The current study was carried out to detect *Salmonella* spp. contamination on chicken carcasses produced at major poultry slaughterhouses in South Korea.

Methods: To determine the seasonal prevalence, serotypes, and antibiotic resistance patterns of *Salmonella*, a total of 120 chicken carcasses were collected through twelve individual samplings (20 chickens per month) in summer 2014 and winter 2015.

Results: A total of 18 chicken samples (15%) were found to be contaminated with *Salmonella*, with a higher rate of contamination observed during summer (14 isolates, 11.7%) than during winter (4 isolates, 3.3%). Among these isolates, *S. enterica* serotype Typhimurium was the most prevalent, followed by *Salmonella* Hadar, Bareilly, and Virchow. A single strain among 5 MDR isolates was resistant to 10 antibiotics, including third-generation cephalosporins. This cephalosporin-resistant strain exhibited the extended-spectrum β-lactamase phenotype and harbored the gene encoding CTX-M-15, the most prevalent ESBL enzyme worldwide. Based on molecular subtyping using an automated rep-PCR system (DiversiLab), all *Salmonella* isolates except the ESBL-producing strain showed low genetic heterogeneity, with more than 95% similarity in their rep-PCR banding patterns. The ESBL-producing isolate was distinguished by molecular subtyping patterns and distinct antibiotic resistance profiles.

Significance: Given that poultry slaughterhouses are considered the last stage in the chicken production chain, the occurrence of *Salmonella* including ESBL-producing strains in individually packaged chickens highlights the necessity for regular monitoring of *Salmonella* strains in poultry slaughterhouses.

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P3-147 Novel Lactic Acid Bacteria (L14 and L28) as a Biocontrol Agent for Inhibition of *Salmonella* in a Raw Chicken Fat Used as a Dog Food Ingredient

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Undergraduate Student Award Competitor

Introduction: Chicken fat being a rich energy source has many important functions in the canine and feline diet. It is often used as a coating for pet food kibble. However, *Salmonella* is a major pathogen in poultry products and is a frequent vehicle of these bacteria thus posing a risk to pet food.

Purpose: The objective of this study was to evaluate the effect of novel isolated lactic acid bacteria (LAB) (L14, L28) on reducing the amount of *Salmonella* in raw chicken fat stored at room temperature.

Methods: For both control and treatment groups, approximately 40 ml of chicken fat was inoculated with a 3-strain *Salmonella* cocktail (Typhimurium, Enteritidis and Newport) for a final concentration of log 3.00 CFU/ml. Each treatment group received respective treatments of L14 or L28 for a final concentration of log 6.00 CFU/ml. The 40 ml chicken fat was aliquot by 10 ml for each time point and was stored at 25ºC. The chicken fat was enumerated on day 0, 1 and 3 on Xylose Lysine deoxycholate (XLD) agar.

Results: After 1 day there were statistical significant differences between the control and the treatments for counts of *Salmonella*. After 1 and 3 days the *Salmonella* in the control chicken fat had grown to approximately log 5.49 CFU/ml and log 7.13 CFU/ml, respectively. For the L14 treatment on day 3, there was a 4.06-log reduction of *Salmonella*. Moreover, on day 3 for L28 treatment there was a 7.13-log reduction and not detectable by means of direct agar plating method.

Significance: Pets that consume contaminated pet kibble can be colonized with *Salmonella* organisms without exhibiting clinical signs, making the pet a possible source of contamination to people in the household. LAB can be provided to processors in various forms (frozen, liquid or freeze-dried).

P3-148 Mechanisms of Inhibition of *Salmonella* by Lactic Acid Bacteria Cocktail (NP51, NP28, NP7, NP3)

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Introduction: Lactic acid bacteria (LAB) are generally regarded as safe by the FDA. LAB has proven effective at inhibiting foodborne pathogens (Shiga toxin-producing *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*) in culture media and/or food products.

Purpose: The objective of this study is to evaluate the mechanisms of a 4-strain cocktail of lactic acid bacteria (NP51, NP28, NP7 and NP3) against a 3-strain cocktail of *Salmonella* (Typhimurium, Enteritidis and Newport).

Methods: *Salmonella* was subjected to treatment with a LAB freeze-dried cocktail and washed LAB cells. Treatments were altered by adding enzymes to determine whether the mechanism of *Salmonella* inhibition is caused by a protein-based antimicrobial compound. A control of *Salmonella* was inoculated in MRS broth; additionally, a treatment of *Salmonella* co-inoculated with LAB product, without enzyme was evaluated for each respective temperature. Samples were stored at 7°C and 37°C and analyzed for viable cell counts of *Salmonella* and LAB on days 0 and 5 for 7°C and hours 0 and 24 for 37°C.

Results: For both LAB products (washed cells and freeze-dried product) held at 37°C, with and without enzyme there were significantly ($P<0.05$) less *Salmonella* (approximately log 6.0 CFU/ml) when compared to the control. The difference between control and treatments carried with and without enzyme at 7°C after 5 days were non-significant. These results suggest that (1) Protein-based compounds are not responsible for inhibition and (2) temperature is a factor for *Salmonella* reduction with more inhibition of *Salmonella* at 37°C than at 7°C.

Significance: Understanding the mechanisms of inhibition can result in optimization of the inhibitory effects. It is apparent that the mechanisms vary depending on the temperature and this should be considered during production and use of the LAB product for microbial pathogen inhibition.

P3-149 Organic Acid Treatment of Beef Trim, Combined with Acidified Sodium Chlorite to Reduce *Salmonella* Encased in Lymph Nodes during Grinding

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Introduction: *Salmonella* within lymph nodes can be a source of contamination for beef trim and ground beef products. Organic acids and acidified sodium chlorite are commonly used as post-harvest interventions to reduce microbial contamination on the surface. However, the use of these interventions during grinding to reduce pathogens protected within lymph nodes has not been previously evaluated.

Purpose: To determine the effectiveness of lactic acid and peracetic acid treatments combined with acidified sodium chlorite (ASC) to reduce *Salmonella* embedded in lymph nodes in beef trim during grinding.

Methods: Subiliac lymph nodes were inoculated with 8 log CFU/g of a cocktail containing rifampicin-resistant derivatives of 6 *Salmonella* strains recognized as the most frequent of clinical significance or isolated from cattle lymph nodes. Beef trim was mixed with the inoculated lymph nodes to reach a final concentration of 4.8 log CFU/g. Organic acids were applied to the trim/node mixture using a six-nozzle sanitizing CHAD spray cabinet. After the first application, the sample was coarsely ground using a meat grinder. The product was then fine ground and an ASC solution was applied through a spray application. Samples were stored at 2-4°C in the dark, and three 1-pound subsamples for each treatment combination were obtained at 1, 24, and 72 h after grinding. Resultant *Salmonella* population were enumerated on selective medium supplemented with rifampicin with a thin layer of a non-selective medium to allow for recovery of injured cells.

Results: All treatments effectively reduced *Salmonella* introduced through contaminated lymph nodes on ground beef products ($P< 0.05$). Peracetic acid + ASC and lactic acid + ASC reduced *Salmonella* by 0.5 and 0.6 log CFU/g by 72 h, respectively. No significant difference between ASC and peracetic acid + ASC was observed ($P=0.23$).

Significance: The use of interventions during grinding can reduce *Salmonella* embedded in lymph nodes in ground beef products.

P3-150 Investigating the Effects of Lactic-Citric Acid (LCA) Blend and Sodium Lauryl Sulfate on the Inhibition of Shiga Toxin-producing *Escherichia coli* (STEC) in Broth System

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Introduction: Food grade organic acids are used to control undesirable microbes in foods and are commonly diluted in water to facilitate application of desired concentrations of antimicrobial agents. Water is a poor wetting agent for hydrophobic environments. In hydrophobic environments

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this problem can be circumvented by use of a food grade processing aid. Sodium lauryl sulfate (SLS) is a surfactant that is generally regarded as a safe food additive.

Purpose: The purpose of this study was to investigate the ability of a lactic-citric acid (LCA) blend and SLS to control the growth of Shiga toxin-producing *Escherichia coli* (STEC) in a broth system.

Methods: Rifampicin-resistant *E. coli* O157:H7 and a cocktail of non-O157:H7 strains were evaluated separately (8.0 log CFU/g). A blend solution of LCA at concentrations of 1%, 2%, and 2.4% and SLS at concentrations of 0.05%, 0.25%, and 0.5% were evaluated individually and in combination. A set of samples were inoculated with *E. coli* O157:H7 while another set was inoculated with a cocktail of non-O157:H7 strains (O26, O45, O103, O11, O121, O145, O104:H4). Samples were stomached and plated onto Sorbitol MacConkey agar infused with rifampicin (100 µg/ml) and incubated at 37°C for 24 h. Three replications were conducted.

Results: The ANOVA showed no individual effects ($P > 0.05$) of the LCA blend or SLS on the control of O157:H7 and the non-O157:H7 cocktail in the BHI broth. Applying the combined SLS (0.05% and 0.5%)) and LCA blend (2.4%) in the broth significantly ($P < 0.01$) reduced the non-O157:H7 by 5 log CFU/g and 2.9 and 4.6 log CFU/g in O157:H7 strains. Increasing the SLS concentration (0.25%) in LCA blend (2.4%) was more effective ($P < 0.01$) on O157:H7, showing 5 log CFU/g reduction.

Significance: This work will assist with providing new information on enhancing the wettability and the exposure of pathogens to antimicrobial treatment.

P3-151 Inactivation of *Salmonella* on Fresh-cut Cantaloupes and Strawberries Using Citric Acid and Ultraviolet-C

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Introduction: Fresh-cut fruits are frequently contaminated with various microorganisms during processing and have been implicated with several foodborne illness outbreaks including salmonellosis. Both ultraviolet-C (UV-C) and organic acids have been reported to reduce microbial loads on fresh produce.

Purpose: This study evaluated the effectiveness of citric acid, UV-C, and a combined citric acid and UV-C treatment for reducing *Salmonella* counts on fresh-cut cantaloupes and strawberries.

Methods: Fresh-cut cantaloupe cubes (pH 6.3) and strawberry halves (pH 3.4) were spot inoculated with 10 µl (10^9 CFU/ml) of a bacterial cocktail containing four serotypes of *Salmonell*a. After drying for 45 min, inoculated fruit samples were treated with 2% citric acid for 10 min, UV-C (1,200 Joules) for 5 min, or 2% citric acid for 10 min followed by UVC (1,200 Joules) for 5 min. To determine the effectiveness of these treatments, viable *Salmonella* counts were determined using Xylose Lysine Deoxycholate agar.

Results: The 2% citric acid treatment did not significantly reduce *Salmonella* counts on the two fruit samples. The UV-C treatment caused about 1.0 log reduction of *Salmonella* on both fruits. Treating cantaloupe samples with citric acid prior to UV-C did not enhance the effect of UV-C on *Salmonella*. However, treating strawberry samples with citric acid first followed by UV-C treatment resulted in >3 log reduction of *Salmonella*.

Significance: The study suggests that the combined treatment of 2% citric acid and UV-C was effective in reducing *Salmonella* on the surface of strawberry and it could be potentially used to inactivate *Salmonella* on other similar fresh cut produce to improve food safety.

P3-152 Influence of Various Physical Stressors on the Efficacy of Five Common Antimicrobials Used in Beef and Poultry Industries to Control *Escherichia coli* O157:H7

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❖ ***Developing Scientist Competitor***

Introduction: During slaughter and fabrication, beef and poultry carcasses are commonly subjected to various physical and chemical interventions. Often, these interventions include application of antimicrobials at concentrations approved by USDA-FSIS. Because stressed microorganisms may exhibit resistance to subsequent stressors, it is important to determine how combinations of these physical and chemical interventions potentially impact microbial survival.

Purpose: The purpose of this study was to determine how subjecting *Escherichia coli* O157:H7 to common slaughter/fabrication stressors prior to antimicrobial exposure impacts the minimum inhibitory concentration (MIC).

Methods: *E. coli* O157:H7 (ATCC 33150) was grown in tryptic soy broth for 18 h at 37°C and then exposed to one of seven stresses: no stress (control), salt, acid, alkaline, heat, cold, and freeze-thaw. Cultures were centrifuged and pelletized cells were adjusted to a 0.5 McFarland standard prior to inoculating Mueller-Hinton broth and dosing into a 96-well microplate containing various concentrations of antimicrobials (lauric arginate, citric acid plus hydrochloric acid, peroxyacetic acid plus acetic acid and hydrochloric acid, lactic acid plus citric acid, and lactic acid). MICs were determined from absorbance value (600 nm) interpretations following 18 h of growth at 37°C.

Results: Statistical analysis of MIC absorbance values indicated a significant ($P \leq 0.05$) difference in absorbance for concentration, as expected. However, no difference ($P > 0.05$) in absorbance values was detected for stress or stress*concentration interaction compared to controls for all antimicrobials.

Significance: Continued prudent use of antimicrobials in concert with other interventions (stressors) is unlikely to reduce antimicrobial efficacy against *E. coli* O157:H7. However, investigation into multiple hurdles (e.g., heat shock plus acid shock), and additional cocktails of various STEC serogroups, is warranted. Furthermore, transformation of absorbance values to quantifiable microbial populations may elucidate differences in antimicrobial efficacy and should be explored.

P3-153 Applicability of Novel Bacteriophage Treatments to Reduce Shiga Toxin-producing *Escherichia coli* on Leafy Greens

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Introduction: Shiga toxigenic *Escherichia coli* (STEC) is a major group of foodborne pathogens, accounting for multiple outbreaks and has also become a threat for the fresh-produce industry. Control of these pathogens remains a challenge even with the adaptation of modern practices and technologies. It is therefore important to develop effective and novel control strategies. Bacteriophage-based treatments could offer a natural, specific, and effective strategy to control STEC.

Purpose: Evaluate the effectiveness of STEC-specific bacteriophages on leafy greens.

Methods: Bacteriophages, isolated from cattle operations, showing lytic activity towards diverse STEC strains were used to prepare antibacterial treatments in phosphate buffered saline (PBS). Leafy greens tested were spinach and romaine lettuce. Prepared leafy green samples were transferred to petri-plates containing moistened filter paper and spot-inoculated with 5 log CFU/ml of either individual strains of *E. coli* O45, O103, O111, O121, O26, or O145 or cocktail of *E. coli* O157:H7. Leaves were spray-treated with phages (8 log PFU/ml) or PBS-control, using airbrush filled with treatment solution. Treated leaves were stored for 72 h at 4°C. Surviving bacteria were enumerated at 0 h, 24 h, and 72 h and data analyzed using one way ANOVA ($P<0.05$).

Results: Leafy greens treated with phage cocktail showed an immediate reduction (1.4-3.0 logs) in *E. coli* O157:H7 populations, compared to the positive or PBS control ($P<0.05$) at 0 h. Similarly, leafy greens contaminated with non-O157 STECs and treated with individual phages, also showed an immediate reduction (1.3 to 3.0 logs) in pathogen populations, compared to the positive or PBS control ($P<0.05$) at 0 h. Phage-treated leafy greens continued to show significant reductions (1.4 to 3.0 logs; $P<0.05$) in both *E. coli* O157:H7 and non-O157 STEC populations at 24 and 72 h, compared to the positive or PBS control.

Significance: Bacteriophages could potentially be used as antimicrobial treatments to reduce or inactivate STEC populations on leafy greens.

P3-154 Biocontrol of Verotoxigenic *Escherichia coli* In Vitro and on Romaine Lettuce Using Lytic Phages at Different Temperatures

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❖ ***Developing Scientist Competitor***

Introduction: Vero toxigenic *E. coli*, especially the serogroups O26, O45, O111, O103, O121, O145 and O157 are recognized to cause enteric disease.

Purpose: To evaluate effectiveness of seven bacteriophages isolated from beef cattle to reduce *E. coli* serogroups O26, O45, O103, O111, O121 and O145 and O157 over a range of temperatures (2 °C, 10 °C and 25 °C) .

Methods: Seven VTEC strains at exponential growth, were treated individually with different dilutions of one of seven lytic phages (phage stocks concentrations 10^7 -10^9 log PFU ml) in a duplicate micro-plate assay at 2, 10 and 25°C. Romaine lettuce leaves were washed and cut into pieces (3 x 3 cm) and spot inoculated with a specific VTEC strain. Phages were individually sprayed on each lettuce piece with contact for 1 h (25°C). VTEC survival was detected using total plate counts on MacConkey agar (n= 100).

Results: Phages showed lytic activity against the seven VTEC to differing degrees. As expected, an interaction existed among phage lytic activity and temperature (p=0.003). All phages were effective at 25°C, however only six were able to kill VTEC at 10 and 2 °C at high concentrations (>10^7 -10^9 log PFU/ml), suggesting the phages were causing lysis from without (lysis effected directly by extracellular supplied agents such as tail-associated lysozyme). On lettuce, five phages targeting O26, O45, O121, O145 and O157 effectively reduced VTEC by more than 90%.

Significance: VTEC phages showed lytic activity at refrigeration temperatures and reduced the number of VTEC on the surface of lettuce. Although concentration of phage needs to be determined for optimal phage activity on lettuce, these results are promising for future application of phages to reduce VTEC on fresh vegetables.

P3-155 Shiga-toxigenic *Escherichia coli* Survival in Commercial Cold-Pressed Fresh Juice and Its Reduction Using Antimicrobial Plant Extracts

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Introduction: Raw cold-pressed juice industry (mainly retailers) is booming in North America. However, in the past, several outbreaks related to contaminated juices have been reported.

Purpose: This study aimed to determine Shiga-toxigenic *Escherichia coli* (STEC) (serotypes O26, O45, O1O3, O111, O121, O145 and O157) capacity to survive in commercial cold-pressed juice and to assess the antimicrobial activities of 4 pure essential oils (EOs) against 7 STEC.

Methods: The antimicrobial activity of EOs and STEC survival was measured using macro and microdilution assays at various pH (4 and 7), temperatures (4 and 25°C), and storage times in-vitro and on fresh juice. Fresh cold-pressed juice was purchased in the local market. The pH of the juice samples ranged from 3.6 to 3.8. The effect of pH and temperature on STEC was tested in vitro in the following treatments: T1, pH 4 at 4°C; T2, pH 4 at 25°C; T3, pH 7 at 4°C; and T4, pH 7 at 25°C and on the juice, at 4 and 25°C, over a 5 day incubation period by quadruplicates (n=1,334).

Results: Five of 7 serotypes survived well in cold-press raw juice for at least 4 days at 4°C and pH 3.5 with no significant ($P>0.05$) reduction in viability. Similar results were observed in-vitro. All tested EOs showed antimicrobial activity against the 7 STEC. Thyme showed the lowest MIC (2 µl/L), while for salvia (SO) the MIC was higher (12-25 µl/L). The data showed that EOs, especially thyme and rosemary can reduce the 7 STEC serotypes to undetectable levels in an acidic mixed vegetable/fruit juice at refrigerated temperatures. It has been shown also that the top even STEC can survive in cold-pressed juices under refrigerated conditions and low pH.

Significance: The tested EOs was effective in reducing STEC numbers in-vitro conditions and in the commercial cold-pressed juice. These results are promising for future application of EOs as natural preservatives in foods.

P3-156 Efficacy of Serial Lauric Arginate (LAE) Applications on Chilled Beef Carcasses, Loins and Manufacturing Trim Prior to Grinding for Reducing Surrogate Shiga Toxin-producing *Escherichia coli* (STEC)

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❖ ***Developing Scientist Competitor***

Introduction: USDA declared seven STEC serotypes adulterants in non-intact raw beef products, establishing the need for validated intervention strategies in commercial operations.

Purpose: Evaluate the efficacy of 1) electrostatically applied lauric arginate (LAE) on chilled beef carcasses, 2) LAE applied to subprimals prior to vacuum packaging, followed by a peroxyacetic acid (PAA) spray after bag opening, and 3) LAE spray onto beef trim prior to grinding for reducing STEC surrogates.

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Methods: Four chilled carcass sides were inoculated with a 5-strain surrogate STEC cocktail (5 log CFU/cm^2) and held (~1°C) for 12 h prior to applying 200 ml of 25% LAE solution electrostatically. Loins from these carcasses were treated with LAE (200 ppm/weight) or water using the Sprayed Lethality in Container (SLIC) method prior to vacuum packaging and storage at 4°C. Bags were opened and loins were sprayed with PAA (200 ppm; 45°C). Two trim batches from each carcass side were treated with LAE (200 ppm/weight; 1 min) or water in a tumbler and held for 24 h at 4°C prior to grinding. Ground portions were taken from each batch and held for 2 or 4 days at 4°C. STEC surrogate populations were enumerated after each processing phase or storage period.

Results: Surrogate populations were reduced ($P \leq 0.05$) ~0.7 log CFU/cm^2 on chilled carcass surfaces following the electrostatic LAE application. Reductions of ~1.0 log CFU/cm^2 were observed on LAE treated loin samples after refrigerated holding, with no additional reductions from the PAA spray after bag opening. LAE-treated trim showed a ~0.6 log CFU/g surrogate reduction compared to no reduction for water treated controls.

Significance: Low-volume LAE electrostatic spray application to chilled carcass sides, followed by applications (200 ppm/meat weight) to chilled subprimals and trim/ground beef effectively reduced STEC surrogate populations and would offer processors an additional level of STEC control following primary hot carcasses interventions.

P3-157 Effects of a Novel Compound on the Cytotoxic Activity of Shiga Toxin-producing *Escherichia coli* O157:H7

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Introduction: We have identified a novel compound capable of inhibiting various virulence gene expressions in *Escherichia coli* O157:H7, which could help mitigate its pathogenicity at certain time points in its infection.

Purpose: This study aimed to investigate the effect of compound CCG-203592 on human kidney (HK-2) and intestinal carcinoma (Caco-2) cells based on the influence of the compound on the expression of major virulence genes in *E. coli* O157:H7 strains.

Methods: According to our previous study, virulence gene expression in these strains was significantly decreased after a 6.5-h exposure to CCG-203592. HK-2 and Caco-2 cells were exposed to different concentrations of cell-free extracts or live cells following treatment of the cells with CCG-203592 or DMSO for 6.5 h. After a 3-day incubation, mammalian cell viability was measured by the MTT assay.

Results: Compared with the control, the relative viability of HK-2 cells exposed to the cell-free extract of strain C7927 pre-treated with CCG-203592 or DMSO, at all tested concentrations, was significantly decreased by 80% ($P \leq 0.03$). The viability of HK-2 cells incubated with strain G5101, pre-treated with undiluted and 2× diluted CCG-203592 or DMSO, decreased by 40% ($P \leq 0.1$). For the cell–free extract treatment, approximately 40% ($P \leq 0.1$) of the HK-2 cell viability remained. No significant difference in HK-2 cell viability was seen for either bacterium treated with CCG-203592 and DMSO. No significant cytotoxicity was detected on Caco-2 cells at all concentrations for both strains.

Significance: It is possible that higher concentrations of CCG-203592 are needed to show effectiveness on the expression of the virulence genes. More studies are currently being conducted to further understand and investigate the effectiveness of the compound on *E .coli* O157:H7 virulence.

P3-158 Impact of Food Disinfectants on Formation of VBNC Cells in *Salmonella*

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Introduction: In modern-day industries food products are often subjected to chemical interventions aimed at reducing microbiological hazards. However, many bacterial species including major foodborne pathogens such as *Salmonella* have evolved ways to evade stressful environmental conditions including exposures to potentially lethal chemicals by entering a so called Viable But Non-Culturable (VBNC) state.

Purpose: The goal of this study was to evaluate the impact of commercial food disinfectants on formation of the VBNC state in *Salmonella*.

Methods: A laboratory-based spray model was used to test *Salmonella enterica* bearing chicken parts sprayed with the following disinfectants: acetic acid (AA), citric acid (CA), lactic acid (LA), peracetic acid (PAA), hydrogen peroxide (H_2O_2), or 1, 3-Dibromo-5, 5-dimethylhydantoin (DBDMH). The reduction of *Salmonella* for each test biocide was enumerated by conventional culture plate methods coupled with the direct viability assessment via the LIVE/DEAD fluorescent dyes.

Results: The results show that all the disinfectants with the exception to DBMHD generated up to 1.16, 0.81, 0.46, 0.43, and 0.31 log VBNC *Salmonella* cells in PAA, H_2O_2, CA, LA, and AC treated samples, respectively. The viability of the VBNC cells was further confirmed by a series of resuscitation experiments in which the CA, LA and AC induced VBNC cells were recovered on culture plates by overnight incubation in the low oxygen environment (10% CO_2, 5% O_2, balance N_2). The PAA induced VBNC cells were resuscitated with brief co-incubation with catalase solution (2,000 units/ml).

Significance: Our studies show that some commercially used food disinfectants can induce formation of the VBNC state in *Salmonella*. These VBNC cells did not grow on nutrient rich media while remaining viable and potentially infectious.

P3-159 The Effectiveness of Leucocin A to Inhibit *Listeria monocytogenes* on Ready-to-Eat Meat in the Presence of an Autochthonous Spoilage Organism *Brochothrix thermosphacta*

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Introduction: *Leuconostoc gelidum* UAL187 produces a bacteriocin, leucocin A, which is active against meat spoilage organisms and *Listeria monocytogenes.* However, previous work suggested that leucocin A may be degraded in food.

Purpose: To determine if spoilage organisms have the ability to degrade purified leucocin A and decrease the inhibitory effect against *L. monocytogenes.*

Methods: A number of spoilage organisms, including *Brochothrix thermosphacta*, were screened for their ability to degrade leucocin A *in vitro*. Leucocin A was either partially or fully purified by column chromatography and HPLC. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was used to confirm the presence of leucocin A. Antimicrobial activity was confirmed by spot-on-lawn assay against *Carnobacterium divergens* UAL9. To confirm degradation in food, hot dogs with 1 mM leucocin A were co-inoculated with *B. thermosphacta* or *C. divergens* and *L. monocytogenes*, vacuum packaged and stored at 7°C for 2 weeks prior to sampling for antimicrobial activity and peptide degradation via mass spectrometry.

Results: After either partial or complete purification, activity was detected against *C. divergens* UAL9 and MALDI-TOF confirmed the presence of the leucocin A with a mass of 3930 Da. When partially purified leucocin A was combined with a culture of *B. thermosphacta*, no activity was detected against *C. divergens,* but the positive control did provide activity. Similar results were found on hot dogs.

Significance: This research suggested that degradation of bacteriocins by spoilage organisms may be a concern for the application to control the growth of pathogens in food.

P3-160 Prevention of Mixed-species Biofilm Formations on Stainless Steel and Plastic Surfaces by a Nanoscale Plasma Coating

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Introduction: Mixed-species biofilms are more stable than single species biofilms because cell-to-cell interactions can affect their formation. Cold plasma is a novel non-thermal food processing technology that uses energetic, reactive gases to inactivate contaminating microbes on food and food contact surfaces. Nanoplasma coatings can inhibit initial cell adhesion to surfaces by changing the surface energy and contact angle of the surface.

Purpose: To investigate the anti-biofilm efficacy of trimethylsilane (TMS) and TMS+oxygen plasma coatings on stainless steel (SS) and high density polyethylene (HDPE) surfaces against complex biofilms.

Methods: SS and HDPE wafers were coated with TMS and TMS+O_2 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 biofilms were removed by rinsing and ultrasonicating each wafer four times for 30 s each time. The pour-plate method was conducted to determine bacterial counts.

Results: On coated SS, the total number of a mixed-species biofilm of LM and SE dropped from 10^7 (100:1 LM:SE) to 10^6 CFU for both TMS (8:3 LM:SE) and TMS+O_2 (14:3 LM:SE) treatments. EC and SE mixed-species biofilm was reduced by 70% on TMS plasma-coated (1:60 EC:SE) and 45.56% on TMS+O_2 coated (1:30 LM:SE) SS. The LM and SE mixed biofilm was reduced by 1 log CFU on TMS plasma-coated (100% SE) and by 2 log CFU on TMS+O_2 coated (93.3% SE) HDPE. However, there was no significant reduction in EC and SE mixed-species biofilm cell counts on both TMS and TMS+O_2plasma coated HDPE.

Significance: This study shows that TMS-coated SS and HDPE surfaces could significantly inhibit some mixed-species biofilms. Further, the bacteria in a mixed biofilm also played a role in the reduction of a single species in the biofilm.

P3-161 Anti-listerial and Anti-staphylococcal Action of a *Lactococcus lactis* Strain Isolated from Brazilian Fresh Minas Cheese

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Introduction: Lactic acid bacteria (LAB) produce substances that have antagonistic activity against undesired food microorganisms and are of great interest to food industry due to its huge potential as natural food preservatives.

Purpose: In this study, the bioprotective activity of a bacteriocinogenic LAB, isolated from Brazilian fresh Minas cheese and identified by 16S rRNA gene sequencing as *Lactococcus lactis* (strain 11), was evaluated.

Methods: Strain 11 was co-inoculated with food pathogens *Listeria monocytogenes* ATCC 7644 or *Staphylococcus aureus* ATCC 25923 in pasteurized milk, maintained at 8°C for up to 10 days. *Lactobacillus sakei* ATCC 15521 was used as negative control for bacteriocin production (LAB bac$^-$).

Results: After incubation period, monocultures of *L. monocytogenes* reached 8 log CFU ml^{-1}. In the presence of LAB bac$^-$, *L. monocytogenes* population achieved 7.3 log CFU ml^{-1}. However, when co-inoculated with strain 11, *Listeria* counts were maintained at the initial inoculum levels, not surpassing 2.3 log CFU ml^{-1}. Regarding to *S. aureus*, in the end of the experiment, cultures counts were 5.4 log CFU ml^{-1} (monocultures), 5.5 log CFU ml^{-1} (co-inoculation with LAB bac$^-$) and 4.7 log CFU ml^{-1} (co-inoculation with strain 11). Even though growth inhibition of *S. aureus* in the co-inoculation studies was not as evident as *L. monocytogenes* inhibition, *S. aureus* growth was significantly affected (P<0.005, according to Scott-Knott test) by the presence of strain 11.

Significance: These results indicate that *Lactococcus lactis* strain 11 has potential for use as a biopreservative culture in dairy products. Additional studies are being planned to confirm this assumption.

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P3-162 Using a Surfactant to Improve the Efficacy of Antimicrobials against *Salmonella* Attached to Chicken Skin

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❖ ***Developing Scientist Competitor***

Introduction: The topography of chicken skin changes during scalding and defeathering processes. The level and nature of *Salmonella* attachment to skin can therefore determine the efficacy of antimicrobials applied during the latter stages of slaughter.

Purpose: Assess the effect of combining sodium dodecyl sulfate (SDS) with chlorine (Cl) and peracetic acid (PAA) on antimicrobial activity against *Salmonella* on chicken skin defeathered following different scalding temperatures.

Methods: Chicken skins were prepared by 1) no scalding and dry hand-defeathering, 2) tap water (20°C) scalding, mechanically defeathering, 3) soft (51°C) scalding, mechanically defeathering, and 4) hard (60°C) scalding, mechanically defeathering. Skin samples (5 cm diameter) from broiler breast skin were inoculated with nalidixic acid resistant strains of *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Heidelberg as a cocktail. The inoculum was 10^7 CFU/sample. After 10 min attachment period, skins were treated with antimicrobial solutions of 0.005% Cl, 0.2% PAA, 0.5% SDS, 0.005% Cl with 0.5% SDS, and 0.2% PAA with 0.5% SDS. Positive control was used to determine the real inoculum level. Treated skin samples were rinsed with 10 ml of buffered peptone water (BPW) for 1 min first to remove "loosely attached" cells, and skins were then transferred to fresh BPW and stomached for 1 min to remove "firmly attached" cells. All rinsed and stomached samples were plated on Trypticase soy agar plates with nalidixic acid for bacterial enumeration.

Results: There were no differences in *Salmonella* attachment to various chicken skins prepared in this study (P>0.05). The results also showed PAA was more effective against *Salmonella* than Cl, especially on dry defeathered skin. SDS enhanced the efficacy of Cl, but not PAA used in this study.

Significance: Differences in *Salmonella* attachment to various types of chicken skin could not be demonstrated in this study. Therefore, the impact of SDS could not be assessed effectively.

Poster

P3-163 Optimization and Strain Variation for the Reduction of *Salmonella enterica* by Chitosan Microparticles

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❖ ***Developing Scientist Competitor***

Introduction: Contamination of agricultural waters by *Salmonella enterica* presents a challenge to the food industry. Chitosan microparticles (CM) have shown broad-spectrum antibacterial activity against numerous species, including *S. enterica;* however, various environment parameters may alter the potential for its application to food protection.

Purpose: In this study anti-*Salmonella* activity of CM was determined for different CM concentrations and various environmental conditions in order to optimize its efficacy. Strain variation in CM sensitivity was examined, and genomic comparisons investigated genes and genotypes and their relationship to CM sensitivity.

Methods: Growth and survival of a three-strain *S. enterica* cocktail was determined by mean log CFU/ml ± standard deviation in water, pond water, artificial seawater (20 ppt), and nutrient broth with or without addition of various concentrations of CM at different pH (5,7,9) and temperature (25, 30, 37°C) combinations. Survival of various *S. enterica* strains and serotypes was compared under optimized conditions, and genomic sequences of these strains were processed by Bowtie, and Rapid Annotation Subsystem Technology in order to determine phylogenies and annotation of contig files.

Results: Optimum conditions for CM activity against *S. enterica* in water were pH7 and 37°C, but the effects of CM were significantly (P<0.0001) diminished by addition of NaCl, the presence of complex bacterial communities in pond water, and in stationary compared to log phase growth. *S. enterica* strains were non-detectable after 2 h exposure to 0.3% CM in sterile water, and significant reductions were seen with CM concentrations as low as 0.01% (P<0.001). Strains differences in CM sensitivity were observed and varied within serotype, while corresponding genotypes and predicted proteins were associated with increased sensitivity to CM.

Significance: This study provides conditions that may be useful for the application of CM as a sanitizer for irrigation and agricultural wash water.

P3-164 Effectiveness of Wash Water Containing Plant Antimicrobials against *Salmonella* Newport on Organic Leafy Greens during Reuse

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Introduction: Compounds derived from plant sources such as essential oils and plant extracts have shown antimicrobial effects against foodborne pathogens. These plant antimicrobials can be used as alternatives for chemical sanitizers. To reduce the costs associated with post-harvest washing, a common practice in the produce industry is to reuse the wash water.

Purpose: The objective was to investigate the efficacy of wash water containing plant antimicrobials against *Salmonella* Newport on organic romaine and iceberg lettuces and baby spinach during reuse.

Methods: Three types of organic leafy greens- romaine lettuce, iceberg lettuce or baby spinach samples were immersed into 200 ml of 10^6 CFU/ml *Salmonella* Newport culture for 2 min, and dried in a biohood for 30 min. Plant extracts (olive or apple extract) and essential oils (oregano or cinnamon oil) washes were prepared at 3% and 0.3% concentrations, respectively. Produce samples were immersed into wash water for 2 min, and stored in stomacher bags at 4°C. The wash water was reused 5 times to treat five batches of leafy green samples one after another. Produce samples were taken at day 0, 1, and 3 to enumerate *Salmonella* Newport survivors. Wash water samples were also taken after each wash to enumerate *Salmonella* population. Phosphate buffered saline was used as a control.

Results: For olive and apple extract treatments, the *Salmonella* log reductions were 0.5-2.0, 0.5-2.6 and 0.8-2.7 at day 0, 1 and 3, respectively. The 5-time reuse of 3% olive and apple extracts did not reduce their antimicrobial effects. Oregano and cinnamon oil treatments caused 1.5-3.4, 1.4-3.4 and 0.8-3.4 log reductions at day 0, 1 and 3, respectively. There were no *Salmonella* survivors detected in olive extract, oregano, and cinnamon oil wash waters after each wash.

Significance: The results demonstrated that wash water with plant antimicrobials can be recycled to reduce the costs for produce industry.

P3-165 Bacteriophage Treatment of *Salmonella* Contamination on Workers' Boots in a Rendering Processing Environment

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❖ ***Developing Scientist Competitor***

Introduction: Workers' boots are considered as one of the re-contamination routes of *Salmonella* for rendered animal meals in the rendering processing environment.

Purpose: This study was conducted to test the efficacy of bacteriophage cocktail for reducing *Salmonella* contamination of workers' boots and ultimately for preventing *Salmonella* re-contamination of rendered animal meals.

Methods: Under laboratory condition, biofilms of *Salmonella* strain 8243 formed on the boots were treated with phage cocktail (9 log PFU/ml) alone, phage + bleach (400 ppm chlorine), and phage + scrubbing for 6 h in a boot bath. SM buffer and bleach alone were used as controls. A field study was conducted by treating workers' boots with the same phage treatments for 1 week in rendering processing environment, in which all treatments were applied at a rate of 3 times per week. Indigenous *Salmonella* populations were swabbed and enumerated on XLT-4, Miller-Mallinson or CHROMagar plates.

Results: Under laboratory condition, *Salmonella* biofilms formed on the boots were reduced by 1.07 log (91.5%), 1.18 log (93.4%), 2.08 log (99.2%) and 1.52 log (97.0%) CFU/boot after treated with phage cocktail alone, bleach alone, phage + scrubbing and phage + bleach for 6 h, respectively. In a rendering processing environment (Ave. 19.3ºC; Ave. relative humidity: 48%), indigenous *Salmonella* populations on workers' boots were reduced by 0.81 log (84.5%), 1.17 log (93.2%), and 1.15 log (92.9%) CFU/boot after treated with phage cocktail alone, phage + scrubbing, and phages + bleach for 1 week, respectively. Our results demonstrated the effectiveness of phage treatments in reducing *Salmonella* contamination on the boots in both laboratory and rendering processing environment.

Significance: Control of *Salmonella* contamination on workers' boots can reduce the chances for the finished animal meals being re-contaminated with *Salmonella*, and therefore ensure the microbiological safety of rendered animal meals.

Poster

P3-166 Efficacy of a Food-Grade Mixture of Volatile Compounds to Reduce *Salmonella* Levels on Food Contact Surfaces

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Introduction: Volatile organic compounds (VOCs) released from an endophytic fungus, *Muscodor crispans,* have been shown to have antimicrobial activity against many fungal and bacterial species. These VOCs have been synthesized into a commercial mixture called "B-23," which may be a useful surface sanitizer. All components in B-23 are Generally Recognized as Safe (GRAS) substances, making B-23 potentially useful as a food contact sanitizer.

Purpose: This study determined if the sanitizing capability of B-23 is equivalent to commonly used sanitizers for reducing *Salmonella* contamination levels on food contact surfaces.

Methods: Coupons of food contact material (stainless steel, PVC conveyor belt material, HDPE cutting board material, and HDPE tote plastic) were inoculated with a *Salmonella* cocktail either by spot-inoculation or by submerging the coupons in a *Salmonella* suspension and allowing cells to attach to the surfaces over a 48-h period. For each sample, a disposable wipe was saturated with a sanitizing solution (1% B-23, 200 ppm chlorine, 200 ppm quaternary ammonium compounds, or 200 ppm peracetic acid) and wiped across the surface of a coupon in a consistent manner using the swiper automated machine. *Salmonella* counts on the coupons before and after the treatments were compared to controls to determine the effectiveness of each sanitizer.

Results: The effectiveness of using wipes with B-23 was similar to the other sanitizers in reducing *Salmonella* levels on all food contact surfaces (P>0.05). Coupons that were spot inoculated with *Salmonella* and wiped with B-23 showed a larger reduction (~2.1 log) than coupons that had attached cells (~0.8 log).

Significance: Results showed that a 1% B-23 solution may have similar effectiveness as other common sanitizers when applied with a disposable wipe onto food contact surfaces contaminated with *Salmonella*. B-23 may be an alternative food-grade sanitizer for food contact surfaces since all of its components are GRAS substances.

P3-167 Efficacy Studies of Bromine-based Biocides for the Control of Microorganisms on Pork

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Introduction: Pork meat offers a favorable environment for growth of spoilage organisms and pathogens, therefore antimicrobials or other adequate technologies need to be applied to pork products to ensure safety. Although bromine-based antimicrobials have been widely used in recreational and industrial water treatment for many years, they have only recently been approved for use in some food safety applications. Some advantages of bromine-base biocides are: their effectiveness at a wider range of pH values than chlorine, low odor and minimal effect on organoleptic properties.

Purpose: Evaluate DBDMH (1,3-Dibromo-5,5-Dimethylhydantoin), a bromine-based biocide, as means to control bacteria in the pork industry.

Methods: *Salmonella enterica* was grown overnight at 37°C in Luria-Bertani broth. Pork bellies purchased from a local slaughterhouse were cut into 100 cm^2 pieces, UV-treated for 30 minutes, spot inoculated on muscle or skin side with 6 log CFU/cm^2 of *Salmonella* and allowed attachment for 30 minutes at room temperature. Samples were hung, sprayed from both sides for 15 seconds at 60 PSI for each treatment and allowed to drip for one minute. Treatments included DBDMH 300 ppm (as bromine), peroxyacetic acid (PAA) at 200 ppm or 2% lactic acid. Samples in 0.1% buffered peptone water were rocked, serially diluted and analyzed using standardized methods to detect and quantify *Salmonella* and *Enterobacteriaceae* (EB). Positive and negative controls were handled identically.

Results: DBDMH, PAA and lactic acid reduced *Salmonella* inoculated on skin side by 1.47, 1.47 and 1.38 log CFU/cm^2, and on muscle side by 0.82, 0.82 and 1.09 log CFU/cm^2, respectively. They reduced EB's on skin side by 1.5, 1.48 and 1.45 log CFU/cm^2, and on muscle side by 0.67, 0.74 and 1.25 log CFU/cm^2, respectively.

Significance: DBDMH is an effective intervention to reduce bacteria in the pork industry. Although this study used 300 ppm (as bromine), the FDA's food contact substance notification 1190 allows concentrations up to 900 ppm.

P3-168 Antimicrobial Sensitivity Patterns of Major Zoonotic Pathogens from a Season-long "Farm-to-Fork" Study of All Natural, Antibiotic-free, Pasture-raised Broiler Flocks in the Southeastern United States

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Introduction: The prevalence of antibiotic resistance microorganisms has significant implications for environmental, animal, and human health. One focus is the use of antibiotics in animal agriculture and its effects on antibiotic resistant bacterial populations within those systems, but before this causal effect can be elucidated, a greater understanding of the background/reference levels of antibiotic resistance devoid of antibiotic use is needed.

Purpose: What are the antibiotic resistance profiles of bacteria in agricultural production environments when antibiotics are not used for production purposes? All-natural, pasture-raised production systems where antibiotics are not used nor have been used historically should allow us to better determine background levels of antibiotic resistance in relevant bacteria associated with broiler chicken production.

Methods: Fifteen all-natural, pasture-raised broiler flocks were sampled along the entire "farm-to-fork" continuum, including fecal and soil samples during grow-out, cecal content and carcass rinses during processing, and carcass rinses of the final products delivered to the consumer. Traditional culture methods were used to isolate 3 zoonotic bacterial pathogens (*Salmonella*, *Campylobacter,* and *Listeria*) and generic *Escherichia coli* and their resistance profiles were determined using the CDC's NARMS protocol.

Results: Sensitivities to a variety of antibiotics were found for not only generic *E. coli* isolates, but also for the three zoonotic bacterial pathogens, from various points along the "farm-to-fork" continuum. AST profiles did not appear to be dependent on species/serotype of each pathogen, nor where along the "farm-to-fork" continuum the pathogen was isolated.

Significance: While not surprising, these results show that background levels for resistance in these production systems need to be considered when determining the causal effect of antibiotic use within the production animals to the proliferation of antibiotic resistance organisms.

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